

# APIMONDIA

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APICULTURAL CONGRESS 2023

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*Sustainable Beekeeping, from the south of the world*

## ABSTRACT BOOK

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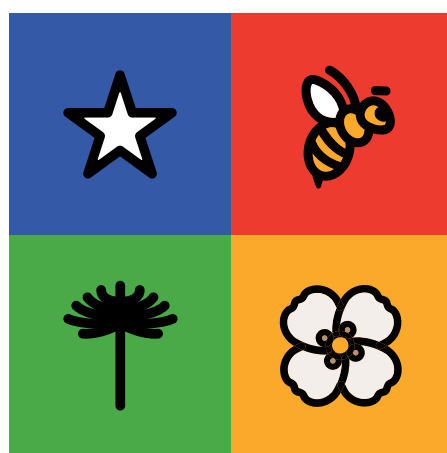
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**APIMONDIA**  
**CHILE 2023**

# ORAL PRESENTATIONS





# ORAL PRESENTATION

## Apitherapy

### OP-001

#### Potential use of propolis as a multitarget therapeutic product: *In Vitro* Evaluation of its immunomodulatory, antibacterial, and anticancer properties

Soumaya Touzani<sup>1</sup>, Walaa Embaslat<sup>2</sup>, Hamada Imtara<sup>3</sup>, Abdalsalam Kmail<sup>3</sup>, Sleman Kadan<sup>3</sup>, Hilal Zaid<sup>3</sup>, Ilham Elarabi<sup>1</sup>, Badiaa Lyoussi<sup>1</sup>, Bashar Saad<sup>3</sup>

<sup>1</sup>Physiology-Pharmacology, University Sidi Mohamed Ben Abdellah, Fez, Morocco

<sup>2</sup>Faculty of Allied Medical Sciences, Arab American University Palestine, Jenin, State of Palestine

<sup>3</sup>Qasemi Research Center, Arab American University Palestine, Jenin, State of Palestine

Propolis is a resin that honeybees produce by mixing saliva and beeswax with exudate gathered from botanical sources. The present in vitro study investigated the potential use of propolis as a multitarget therapeutic product and the physicochemical properties, chemical composition, and immunomodulatory, antioxidant, antibacterial, and anticancer properties of a propolis extract from the northern Morocco region (PNM). Pinocembrin, chrysin, and quercetin were the main phenolic compounds of PNM as measured in HPLC. The PNM showed significant inhibitory effects against all tested Gram-positive and Gram-negative strains and showed high antioxidant activities by scavenging free radicals with IC50 (DPPH=0.02, ABTS=0.04, and FRAP=0.04 mg/ml). In addition, PNM induced a dose-dependent cytostatic effect in MCF-7, HCT, and THP-1 cell lines at noncytotoxic concentrations with IC50 values of 479.22, 108.88, and 50.54 µg/ml, respectively. The production of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) was decreased in a dose-dependent manner in LPS-stimulated human peripheral blood mononuclear cells (PBMCs), whereas the production of the anti-inflammatory interleukin-10 (IL-10) was increased in a dose-dependent manner reaching 15-fold compared to the levels measured in untreated PBMCs. Overall, the results showed that the traditionally known multitarget therapeutic properties of the PNM seem to be mediated, at least in part, through cytostatic, antibacterial, and immunomodulatory effects.

### OP-002

#### Building the BEEARC: The Honeybee and The Human Heart

James Townsend Fearnley

Apiceutical Research Centre

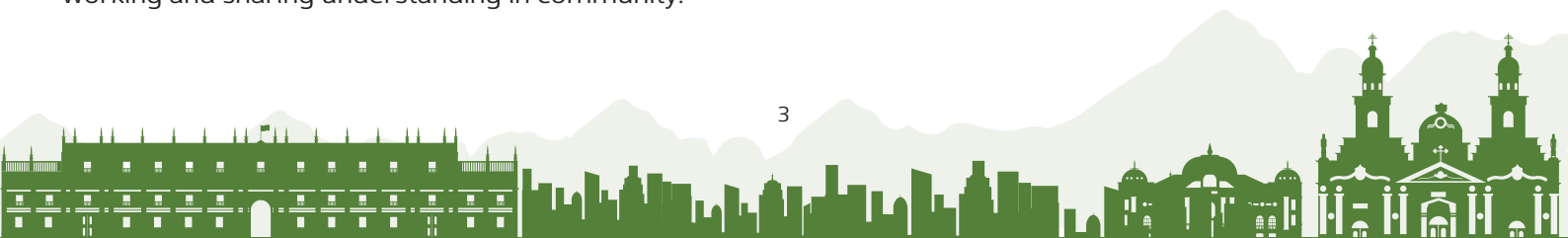
There has been an explosion of research interest in the medicinal properties of products from the beehive over the last 30 years - Propolis, Honey, BeeVenom. The therapeutic use of bee products has moved from the periphery of health treatment into the centre of a new wave of public interest in so called alternative or complementary therapies. Clinical studies highlighting the pharmacological properties of bee products, honey and propolis in particular, are now common place. In some Turkish medical schools doctors are now being trained in BeeVenom therapy. A growing number of research papers are tackling the growing global problem related to pharmaceutical medicine - side effects, iatrogenesis and antibiotic resistance.

James Fearnley has, over the last 30 years been working in different ways to coordinate and develop this new genre of medicines he calls Apiceuticals.

Fearnley created ARC Apiceutical Research Centre in 2011. ARC staged the first conference on Apiceuticals - Medicines of the Future at the University of Strathclyde in 2011. A further international conference focusing on Propolis in Human and Bee Health took place, again at the University of Strathclyde in 2016. Fearnley was responsible for creating the International Propolis Research Group which has attracted scientists from round the world to its annual International Conferences.

Fearnley's personal research journey however - publishing books and over 30 peer review papers - has now led him to the belief that the honeybee can provide human beings not only with nature's most powerful medicines but can inform and inspire and support human beings in other realms essential to our real, holistic and sustainable health.

The BEEARC project is a 7 million pound project to build a three dimensional response to the human being current social, economic and cultural health crisis. The three elements, all inspired by the honeybee, will include, a Discovery Centre exploring the social, economic and cultural meaning of the honeybee, a Research Centre researching sustainable social, economic and cultural developments and a Model Village Community illustrating in practice new ways of living, working and sharing understanding in community.



### OP-003

#### **Inhibitory effects of aqueous ethanol extracts of poplar-type propolis from China on the formation of advanced glycation end products**

Yu Zhang<sup>1</sup>, Jiangtao Qiao<sup>2</sup>, Tongtong Ye<sup>1</sup>

<sup>1</sup>Jiangsu Beevip Biotechnology Co., Ltd, Taizhou, Jiangsu, China

<sup>2</sup>Institute of Apicultural Research, Chinese Academy of Agricultural Sciences

Advanced glycation end products (AGEs), the nonenzymatic glycation between sugar dicarbonyls and proteins, is the key factor of diabetic complication due to hyperglycemia. We hypothesis that propolis extracts has a high anti-glycation potency due to a high concentration of antioxidant phenolics and the anti-glycation of various extracts of propolis were evaluated. Various aqueous ethanol extracts of propolis have strong antioxidation in Glucose autoxidation, especially 75% ethanol extracts of propolis (EEP). In a bovine serum albumin (BSA)-glucose model and BSA-Methylglyoxal (MGO), 75%EEP have the highest inhibitory effects against the total AGE formation (74.05% and 82.09 %, respectively). Such abilities were higher than natural inhibitor rutin (100 $\mu$ M) and 1mM aminoguanidine (AG) solution ( $p < 0.05$ ), a commonly used chemical inhibitor of glycation process. In additional, these extracts were found to be effective in inhibiting the two special AGE, pentosidine and N $\epsilon$ -carboxymethyllysine (CML), compared to or slightly higher than the effect of AG, in which 75% EEP have the highest inhibitory effect. All propolis extracts significantly decreased in their protein carbonyl (PCO), increased in protein thiol levels, and inhibited tryptophan fluorescence quenching. The main components of Chinese propolis were found as phenolics. We concluded that aqueous ethanol extracts of propolis have high anti-glycation potencies, which might be attributed to possessing antioxidative activities and trapping reactive carbonyl species. These results suggest that aqueous ethanol extracts of propolis can be used to develop functional foods and nutraceuticals for inhibiting diabetic complication and relative disease induced by AGE.

### OP-004

#### **Poplar propolis improves insulin homeostasis and pancreatic cell function in pre-diabete mellitus obese volunteers; a crossover randomized controlled trial**

Cardinault Nicolas<sup>1</sup>, Sani Léa<sup>2</sup>, Astier Julien<sup>2</sup>, Darmon Patrice<sup>2</sup>, Landrier Jean Francois<sup>2</sup>

<sup>1</sup>Association Francophone d'Apithérapie, france

<sup>2</sup>Aix-Marseille Université, C2VN, INRAE, INSERM, 13000, Marseille, France

**INTRODUCTION:** Diabetes mellitus represents a major public health problem, with an increasing prevalence, affecting more than 422 million adults in 2014 (OMS 2016). During the natural course of diabetes, concomitantly insulin levels increase with the expansion of adipose tissue in obesity and result in a defect in insulin sensitivity (i.e., insulin resistance), glucose uptake and increased hepatic glucose production, leading to fasting hyperglycemia. In response, there is pancreatic insulin hypersecretion, which defines the asymptomatic phase of prediabetes, resulting in early metabolic abnormalities (Kalin et al., 2017). This asymptomatic phase is crucial because if prediabetes is left untreated, the risk of developing T2DM is 37% over the next few years, whereas lifestyle intervention could reduce this development to 20%. The objective of this study is to evaluate the effect of standardized poplar propolis extract powder (PPEP) on insulin homeostasis in pre-diabetes mellitus obese volunteers.

**DESIGN:** The trial was a randomized, controlled, crossover, intervention study (NCT05717881). Pre-diabetes mellitus obese volunteers (n=9) were subjected to two periods of supplementation (placebo and PPEP) for 3-months. Quantity of PPEP administered was determined individually to reach 6 mg of polyphenols/kg body weight. The primary outcome of the study was insulin sensitivity measured by the Matsuda index (ISI-M).

**RESULTS:** After 3 months of PPEP supplementation area under the curve (AUC) of insulin during oral glucose tolerance test (OGTT) were significantly decreased. Intergroup variations between baseline and post-supplementation (delta) showed a significant decrease in glycated hemoglobin A1c (HbA1c) and insulin AUC post-OGTT in the PPEP group. PPEP supplementation also improved insulin sensitivity, insulin secretion and more indices.

**CONCLUSIONS:** Based on this study, our standardized PPEP supplementation for 3 months in insulin- Pre-diabetes mellitus obese subjects lead to an improvement in insulin homeostasis, insulin resistance, insulin secretion indexes and Hba1c levels. This study suggests that a standardized poplar propolis extract can exhibit a preventive effect on the physiopathological mechanisms and the development of T2DM and therefore, can help to prevent of the disease.



## Apitherapy

### OP-005

#### Guidelines for therapeutical use of beehive air

Stefan Stangaciu

Romanian Society of Apitherapy, Phytotherapy and Aromatherapy

The healing properties of beehive air were first documented in a 1920 Russian journal.

In 1952, Theodore Jachimowicz, a young Austrian beekeeper, experienced a miraculous recovery from his allergic rhinitis on his first day working in the apiary of the Vienna Beekeeping Institute.

A Hungarian beekeeper also reported improved breathing while performing migratory beekeeping in his bee truck in 1953.

Despite Mr. Jachimowicz's personal experiences being shared with many beekeeping communities, including Apimondia conferences, it was not until 1987 when another Austrian beekeeper, Heinrich Hüttner, professionally extracted beehive air for therapy purposes.

Since Hüttner's lecture in Passau, Germany in 2007 (during the German Apitherapy Congress), Beehive Air Therapy (BHAT) has gained global attention and is now widely practiced in Europe and beyond.

BHAT is often coupled with Api-Education, Apitherapy, Api-Cosmetics, and Api-Tourism, and can greatly benefit local communities through the efforts of beekeepers.

As with any treatment, it is crucial to adhere to principles, rules, and regulations to achieve success, avoid adverse reactions, and legal issues. All these rules will be explained, in detail, during the presentation.

### OP-006

#### Propolis as a useful aid for reducing the severity of COVID-19 disease

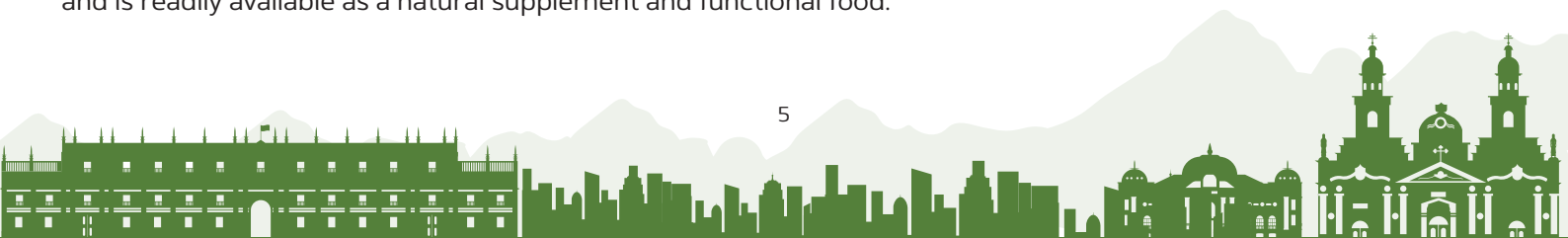
Andresa Aparecida Berretta<sup>1</sup>, David De Jong<sup>2</sup>, Marcelo Augusto Silveira<sup>3</sup>

<sup>1</sup>Research, Development and Innovation Department, Apis Flora Indl. Coml. Ltda., Ribeirão Preto, Brazil

<sup>2</sup>Ribeirão Preto Medical School, University of São Paulo (FMRP/USP), Ribeirão Preto, Brazil

<sup>3</sup>Nephrology Department, Hospital São Rafael, D'Or Institute for Research and Education (IDOR), Salvador, Brazil

Propolis is widely consumed throughout much of the world as a health aid and immune system booster. The COVID-19 pandemic greatly increased the demand for propolis worldwide, especially because of its antiviral properties. COVID-19, caused by infection with SARS-CoV-2 virus causes immune system suppression, and subsequently, lung inflammation and fibrosis. Based on evidence from previous research, including in silico research, it was predicted that propolis can interfere with SARS-CoV-2 attachment to the host cell, viral replication, and pathophysiological consequences of this infection. Pre-clinical studies demonstrated that propolis promotes immunoregulation of pro-inflammatory cytokines, reducing the risk of cytokine storm syndrome, a major mortality factor in advanced COVID-19 disease. There was also considerable evidence that propolis can help ameliorate various comorbidities that greatly increase the risk for mortality in COVID-19 patients, including respiratory diseases, hypertension, diabetes, and cancer. Because of the health emergency during the peak of the COVID-19 pandemic, a clinical trial with a standardized propolis product (EPP-AF), mainly composed of Brazilian green propolis, was authorized for use as an adjunct treatment for 142 hospitalized adult COVID-19 patients in Salvador, Bahia, Brazil, divided into two propolis treatment groups and a control group. Patients consumed 400 mg or 800 mg propolis in capsules during seven days, in addition to the standard treatment regime. Time in the hospital was significantly shortened in the propolis treatment groups compared to the controls. With the higher dose, significantly fewer patients developed acute kidney injury than in the controls. Propolis proved to be a relevant therapeutic option that is safe, easy to administrate orally and is readily available as a natural supplement and functional food.



### OP-008

#### Steps of healing of Qatada (*Acacia hamulosa*) honey on open method of pilonidal sinus

Ahmed Hegazi<sup>1</sup>, Faiz M Al Guthami<sup>2</sup>, Mohamed H. Basiouny<sup>3</sup>, Ahmed Al Gethami<sup>2</sup>

<sup>1</sup>National Research Centre, Dokki, Giza, Egypt

<sup>2</sup>Al Guthami Foundation, Saudi Arabia

<sup>3</sup>Ministry of Health, Egypt

This study aimed to through more light on the effect of Saudi Arabia Qatada (*Acacia hamulosa*) honey sample as a dressing on pilonidal sinus wound healing, antibacterial activity, and cytokines level. The results revealed that the pH of 3.56 of tested honey, where glucose was 378mmol/l also the specific gravity was 1.435, while contents of total phenolic and flavonoid and DPPH activity (32.15 mg GAE/100 g honey), (54.27 mg RE/100 g honey) and (127.83 mg/ml) respectively.

The mean duration of pilonidal sinus wounds to be ready for surgical closure was 43.5 days in the control (standard dressing) group where the honey dressing group was 28.5 days. Less pain, edema, and foul-smelling discharge were expressed in the honey group if compared with the standard dressing group.

The total bacterial count was  $9 \times 10^9$  at the begging of the experiment then it reduced after the application of standard dressing traditional therapy to ( $9 \times 10^5$ ) and honey treatment group ( $9 \times 10^2$ ) after 21 days post-treatment. Significant reduction ( $p < 0.05$ ) was observed in serum TNF $\alpha$  (-52), IL6 - 46), and IL-17 (-60) relatively to pre-treatment values.

From the overmentioned results, it could conclude that Saudi Arabia Qatada (*Acacia hamulosa*) honey bioactivities had been good wound repair due to promoting the growth of tissues and reduction of total bacterial count and inflammation.

### OP-009

#### Protective Effect of Honey and Propolis against Gentamicin- Induced Oxidative Stress and Hepatorenal Damages

Lyoussi Badiia<sup>1</sup>, Hassan Laaroussi<sup>1</sup>, Meryem Bakour<sup>1</sup>, Driss Ousaaid<sup>1</sup>, Pedro Ferreira Santos<sup>2</sup>, Zlatina Genisheva<sup>2</sup>, Asmae El Ghouizi<sup>1</sup>, Abderrazak Aboulghazi<sup>1</sup>, José Antonio Teixeira<sup>2</sup>

<sup>1</sup>Lyoussi Badiia, <sup>1</sup>Laboratory SNA MOPEQ, University Sidi Mohamed ben Abdallah, Fez, Morocco

<sup>2</sup>Hassan Laaroussi, <sup>1</sup>Laboratory of Physiology-Pharmacology and Environmental Health, SNA MOPEQ, University of Sidi Mohammed Ben Abdallah, Fez, Morocco

<sup>3</sup>Meryem Bakour, <sup>1</sup>Laboratory of Physiology-Pharmacology and Environmental Health, SNA MOPEQ, University of Sidi Mohammed Ben Abdallah, Fez, Morocco

<sup>4</sup>Driss Ousaaid, <sup>1</sup>Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life (SNA MOPEQ), Sidi Mohamed Ben Abdallah University, 30000 Fez, Morocco

<sup>5</sup>Pedro Ferreira-Santos, <sup>2</sup>CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

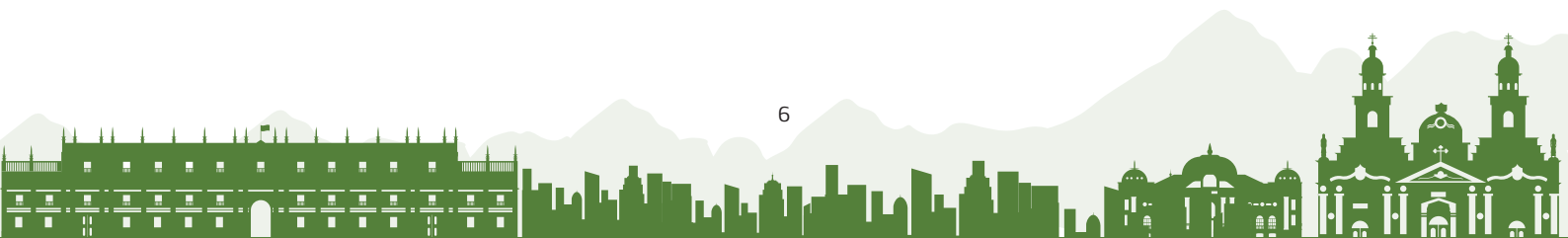
<sup>6</sup>Zlatina Genisheva, <sup>2</sup>CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

<sup>7</sup>Asmae El Ghouizi, <sup>1</sup>Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life (SNA MOPEQ), Sidi Mohamed Ben Abdallah University, 30000 Fez, Morocco

<sup>8</sup>Abderrazak Aboulghazi, <sup>1</sup>Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life (SNA MOPEQ), Sidi Mohamed Ben Abdallah University, 30000 Fez, Morocco

<sup>9</sup>José Antonio Teixeira, AMOPEQ), <sup>2</sup>CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Bee products are a promising source of phenolic compounds with strong antioxidant activity. The present study was designed to explore the protective effect of honey, propolis, and their combination on gentamicin-induced oxidative stress and hepatorenal dysfunction. This study was conducted on male Wistar rats by intraperitoneal injections of gentamicin (120 mg/kg BW/day, i.p.) or normal saline (1 ml/kg BW/day, i.p.) for 10 consecutive days. Honey (2 g/kg BW), propolis (100 mg/kg BW), or their combination were given daily by gavage to normal and gentamicin groups. Honey and propolis samples were evaluated for their phytochemical composition and antioxidant capacity. The in vitro investigations showed that the evaluated samples especially propolis extract have high antioxidant power associated with the presence of several phenolic compounds such as flavonoids, flavan-3-ols, hydroxybenzoic acids, hydroxycinnamic acids, and stilbenes, while honey contains only hydroxybenzoic acids and hydroxycinnamic acids. It was also shown that simultaneous treatment with honey or propolis extract alone or in association prevented changes caused by gentamicin administration and improved hepatic and renal functions. Changes caused by gentamicin administration, observed by in vivo experiments, include significant elevation of uric acid, urea, creatinine, and hepatic enzyme levels (ALT, AST, and ALP) and kidney biochemical changes (an increase of urea, uric acid, and creatinine and a decrease of albumin and total protein) as well as remarkable changes of renal and liver oxidative stress markers (CAT, GPx, and GSH) and elevation of MDA levels. Overall, it can be concluded that honey and propolis might be useful in the management of liver and renal diseases induced by xenobiotics.



## Protective effect of bee pollen in acute kidney injury, proteinuria, and crystalluria induced by ethylene glycol ingestion in rats

Iyousi badiaa<sup>1</sup>, Asmae Elgouizi<sup>1</sup>, Noori Al Waili<sup>2</sup>, Nawal Elmenyiy<sup>1</sup>, Salma El Fetri<sup>1</sup>, Abderrazak Aboulghazi<sup>1</sup>, Ahmed Al Waili<sup>2</sup>, Soumaya Touzani<sup>1</sup>

<sup>1</sup>Iyousi Badiaa, <sup>1</sup>Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health, and Quality of Life (SNAMOPEQ), Sidi Mohamed Ben Abdellah University, 30000 Fez, Morocco.

<sup>2</sup>Asmae Elghouizi, <sup>1</sup>Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health, and Quality of Life (SNAMOPEQ), Sidi Mohamed Ben Abdellah University, 30000 Fez, Morocco.

<sup>3</sup>Noori Al Waili <sup>2</sup> New York Medical Care for Nephrology, Queens, NY 11418, USA

<sup>4</sup>Nawal Elmenyiy <sup>1</sup>. Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health, and Quality of Life (SNAMOPEQ), Department of Biology, Faculty of Sciences Dhar Mehraz, Sidi Mohamed Ben Abdellah University, 30000 Fez, Morocco.

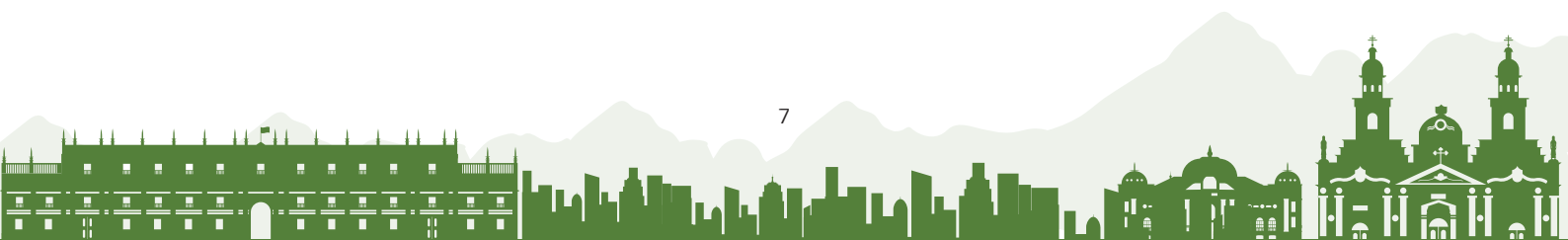
<sup>5</sup>Salma El Fetri, <sup>1</sup>Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health, and Quality of Life (SNAMOPEQ), Sidi Mohamed Ben Abdellah University, 30000 Fez, Morocco.

<sup>6</sup>Abderrazak Aboulghazi, <sup>1</sup>Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health, and Quality of Life (SNAMOPEQ), Department of Biology, Sidi Mohamed Ben Abdellah University, 30000 Fez, Morocco.

<sup>7</sup>Ahmed Al Waili <sup>2</sup> New York Medical Care for Nephrology, Queens, NY 11418, USA

<sup>8</sup>Soumaya Touzani, <sup>1</sup> Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health, and Quality of Life (SNAMOPEQ), Sidi Mohamed Ben Abdellah University, 30000 Fez, Morocco.

Oxidative stress plays a role in hyperoxaluria-induced kidney injury and crystallization. Bee pollen is a hive product with a high content of antioxidants. The antioxidant content and protective effect of bee pollen extract (BPE) against ethylene glycol (EG) induced crystalluria, and acute kidney injury (AKI) were investigated. The effect of BPE on the EG-induced liver injury and proteinuria was also examined. Ten groups of male Wister rats were treated daily with vehicle, cystone, BPE (100, 250, and 500 mg/kg b.wt.), and group 6–9 treated with EG, EG + BPE (100, 250, and 500 mg/kg b.wt.) and group 10 EG + cystone. The dose of EG was 0.75% v/v, and the dose of cystone was 500 mg/kg b.wt. On day 30, blood and urine samples were collected for analysis. Kidneys were removed for histopathological study. The antioxidant activity of BPE was assessed, and its total phenols and flavonoids were determined. EG significantly increased urine parameters (pH, volume, calcium, phosphorus, uric acid, and protein), blood urea, creatinine, and liver enzymes ( $P < 0.05$ ). EG decreased creatinine clearance and urine magnesium and caused crystalluria. Treatment with BPE or cystone mitigates EG's effect; BPE was more potent than cystone ( $P < 0.05$ ). BPE increases urine volume, sodium, and magnesium compared to the control and EG treated groups. BPE reduces proteinuria and prevents AKI, crystalluria, liver injury, and histopathological changes in the kidney tissue caused by EG. BPE might have a protective effect against EG-induced AKI, crystalluria, proteinuria, and stone deposition, most likely by its antioxidant content and activity.





## Systematically Investigating the Protective Role of Standardized Indian Bee Propolis against Inflammatory and Tau-phosphorylation Pathway of Alzheimer's disease

Sandeep Sankaran<sup>1</sup>, Rahul N Dubey<sup>2</sup>, Snehalatha Diddi<sup>3</sup>, Arulmozhi Sathiyarayanan<sup>3</sup>, Sathiyarayanan Lohidasan<sup>2</sup>

<sup>1</sup>Department of Quality Assurance Techniques, Poona College of Pharmacy, Bharati Vidyapeeth Deemed to be University, Pune-411038, India

<sup>2</sup>Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed to be University, Pune-411038, India

<sup>3</sup>Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed to be University, Pune-411038, India

The pathological hallmarks of Alzheimer's disease (AD) involve accumulation of senile plaques and neurofibrillary tangles in the hippocampal, cortex and other surrounding regions of the brain. With current approved treatment regimens only providing symptomatic care for some degree of AD pathology, there arises a need to look at alternatives containing multiple components acting synergistically at multi-targets of AD pathology. Bee propolis is one such product containing diverse class of phytoactive metabolites with ethnopharmacological evidence of its use against complex multifactorial diseases. However, there is minimal awareness amongst Indian beekeepers about its therapeutic potential. Therefore in this study, we aimed to methodically correlate the chemical profile of Indian Propolis (IP) by HPTLC, evaluate in-vivo the anti-inflammatory and neuroprotective effect against okadaic acid mediated memory deficits in Wistar rats and validate the protein targets by molecular docking. The fingerprint of IP standardized to key phenolic markers viz. Pinocembrin, Galangin, Chrysin, Luteolin and p-coumaric acid was achieved using HPTLC complemented by UV-Visible and MS detection systems. Galangin ( $5.78 \pm 0.30$  % w/w) was the major flavonoid present, followed by Pinocembrin ( $2.30 \pm 0.12$  % w/w). The standardized extracts (100, 200, 300 mg/kg) and positive control - Memantine (1 mg/kg) was administered orally for 21 days post once intracerebroventricular (icv) okadaic acid (Oka - 200 ng/rat) induction to animals. The IP treated groups showed improved cognition evidenced by decreased escape latency and distance travelled assessed using the Morris water maze test, increased % reduction in social interaction time and discrimination index by the social and novel object recognition test respectively. On comparison with the disease control group, the biochemical parameters for Oka treated with IP resulted in improved brain acetylcholinesterase activity, protein phosphatase 2A, antioxidant biomarkers, reduced levels of oxidative stress and pro-inflammatory markers, restored brain monoamine amounts and moderately reversed neurofibrillary tangle formation. Additionally, molecular docking of components in IP against tau mediated targets further revealed the potential inhibitory and neuroprotective role. Overall, the findings of the study would increase demand for medicinal Indian propolis as a neuroprotective alternative and more importantly help beekeepers with additional revenue source.

## Apitherapy

### OP-011

## Systematically Investigating the Protective Role of Standardized Indian Bee Propolis against Inflammatory and Tau-phosphorylation Pathway of Alzheimer's disease

Sandeep Sankaran<sup>1</sup>, Rahul N Dubey<sup>2</sup>, Snehalatha Diddi<sup>3</sup>, Arulmozhi Sathiyarayanan<sup>3</sup>, Sathiyarayanan Lohidasan<sup>2</sup>

<sup>1</sup>Department of Quality Assurance Techniques, Poona College of Pharmacy, Bharati Vidyapeeth Deemed to be University, Pune-411038, India

<sup>2</sup>Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed to be University, Pune-411038, India

<sup>3</sup>Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed to be University, Pune-411038, India

The pathological hallmarks of Alzheimer's disease (AD) involve accumulation of senile plaques and neurofibrillary tangles in the hippocampal, cortex and other surrounding regions of the brain. With current approved treatment regimens only providing symptomatic care for some degree of AD pathology, there arises a need to look at alternatives containing multiple components acting synergistically at multi-targets of AD pathology. Bee propolis is one such product containing diverse class of phytoactive metabolites with ethnopharmacological evidence of its use against complex multifactorial diseases. However, there is minimal awareness amongst Indian beekeepers about its therapeutic potential. Therefore in this study, we aimed to methodically correlate the chemical profile of Indian Propolis (IP) by HPTLC, evaluate in-vivo the anti-inflammatory and neuroprotective effect against okadaic acid mediated memory deficits in Wistar rats and validate the protein targets by molecular docking. The fingerprint of IP standardized to key phenolic markers viz. Pinocembrin, Galangin, Chrysin, Luteolin and p-coumaric acid was achieved using HPTLC complemented by UV-Visible and MS detection systems. Galangin (5.78±0.30 % w/w) was the major flavonoid present, followed by Pinocembrin (2.30±0.12 % w/w). The standardized extracts (100, 200, 300 mg/kg) and positive control - Memantine (1 mg/kg) was administered orally for 21 days post once intracerebroventricular (icv) okadaic acid (Oka - 200 ng/rat) induction to animals. The IP treated groups showed improved cognition evidenced by decreased escape latency and distance travelled assessed using the Morris water maze test, increased % reduction in social interaction time and discrimination index by the social and novel object recognition test respectively. On comparison with the disease control group, the biochemical parameters for Oka treated with IP resulted in improved brain acetylcholinesterase activity, protein phosphatase 2A, antioxidant biomarkers, reduced levels of oxidative stress and pro-inflammatory markers, restored brain monoamine amounts and moderately reversed neurofibrillary tangle formation. Additionally, molecular docking of components in IP against tau mediated targets further revealed the potential inhibitory and neuroprotective role. Overall, the findings of the study would increase demand for medicinal Indian propolis as a neuroprotective alternative and more importantly help beekeepers with additional revenue source.

### OP-012

## Development of an apitoxin-based solution for topical use in canines

Catherine Fagundez<sup>1</sup>, Romina Pereira<sup>1</sup>, Enrique Nogueira<sup>2</sup>

<sup>1</sup>Pharmacy of Veterinary Hospital Center, Department of Clinic and Veterinary Hospital, Faculty of Veterinary Medicine, UdelaR, Montevideo, Uruguay

<sup>2</sup>Beekeeping, Department of Animal Production, Faculty of Veterinary Medicine, UdelaR, Montevideo, Uruguay

The bee venom is a secreted product from worker and queen bees, widely in demand and well valued. The therapeutic and cosmetic benefits of apitoxin are widely known, and it can find a wide variety of products with this mixture as an active ingredient on the market in homeopathic form for human use. It has been used for hundreds of years to desensitize allergic patients and mainly as an anti-inflammatory and analgesic in joint and periodontal diseases. Despite these benefits, there are no products on the market with this active principle for veterinary use, many times some human pharmaceutical products are used for this purpose. Therefore, in this work, we present the results obtained in the design and develop a topical 1% bee venom based aqueous liquid formulation for anti-inflammatory and analgesic use in canines. Then characterize the formulation obtained according to its physical, chemical, and organoleptic properties. For the development of this formulation, first we perform a study of solubility with different solvents and mixtures of them, and stability of the solution at different pH. In this case we found that the apitoxin has good solubility in water, and in mixtures of ethanol, propylene glycol and glycerin water. Regarding the stability in solution at different pH, short-term stability was observed for pH between 4 and 7. Then we study different mixtures of aqueous-based solvents components of the formulation was carried out looking for a homogeneous and stable mixture. Solvents such as propylene glycol, glycerin, ethanol, liquid vaseline, almond oil and tween 20 were used in this study in different proportions until reaching an adequate mixture. We arrive at a composition of solvents water:propyleneglycol:glycerin:tween 20:apitoxin (68:15:15:1:1) At this time, we are beginning to carry out studies on the stability and organoleptic characterization of the formulation obtained, for which we do not yet have results. In conclusion, it was possible to develop an aqueous liquid formulation for topical application based on 1% apitoxin for use in canines, but stability and organoleptic results are expected.



## Apitherapy

### OP-013

#### Improved Cognition and Neuroprotection of Indian Royal Jelly in Okadaic Acid Model of Alzheimer's Disease

Sathyanarayanan Lohidasan<sup>1</sup>, Rahul N Dubey<sup>1</sup>, Lakshmi Rao<sup>2</sup>, Sandeep Sankaran<sup>3</sup>, Arulmozhi Sathyanarayanan<sup>4</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University) Pune, Maharashtra, India.

<sup>2</sup>Former Assistant Director II, Central Bee Research and Training Institute (CBRTI), Pune - 411016, India

<sup>3</sup>Department of Quality Assurance Techniques, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University) Pune, Maharashtra, India

<sup>4</sup>Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University) Pune, Maharashtra, India

Royal jelly is the superfood consumed lifelong by the queen bee, responsible for its longevity. It holds great medicinal and nutraceutical value as an anti-inflammatory, and antioxidant, proven effective in various complex diseases. Indian Royal Jelly (IRJ) is one of the superior-quality royal jelly as a nutritional product, however, there is minimal evidence of its nootropic action. Therefore, our study aimed to scientifically validate the neuroprotective effect of IRJ in-vivo in the okadaic acid (OKA)-induced Alzheimer's disease (AD) model in Wistar rats and understand the underlying mechanism involved. OKA was intracerebroventricularly (icv) administered and from day 7 was treated orally with IRJ (test) or Memantine (standard) for 21 days. The locomotor activity, spatial and recognition learning, and memory were evaluated periodically by the actophotometer, the Morris water maze (MWM), and the novel object recognition test (NORT), respectively. Biochemical estimations of oxidative stress markers, neuroinflammation, the cholinergic system in the brain, and in-silico docking against tau protein kinases were performed to understand the mechanism of action of IRJ on reversing AD pathologies. In OKA-induced rats, IRJ-treated groups decreased the escape latency and path length in MWM and increased the exploration time for novel objects and the discrimination index in NORT. IRJ was not associated with any changes in locomotor activity. The biochemical assessment showed that IRJ decreased free radical generation (levels of nitric oxide and malondialdehyde) and proinflammatory cytokines and restored the level of free radical scavengers, viz. glutathione, superoxide dismutase, and catalase, in ICV-OKA rats. IRJ increased the level of acetylcholine and inhibited acetylcholinesterase. Moreover, molecular docking studies revealed the potent inhibitory effect of IRJ on the GSK-3 $\beta$  enzyme. The nootropic and neuroprotective actions of IRJ were found comparable to that of Memantine with the findings of the study providing evidence to be effectively explored as a novel therapeutic agent for the treatment of AD and related dementias.

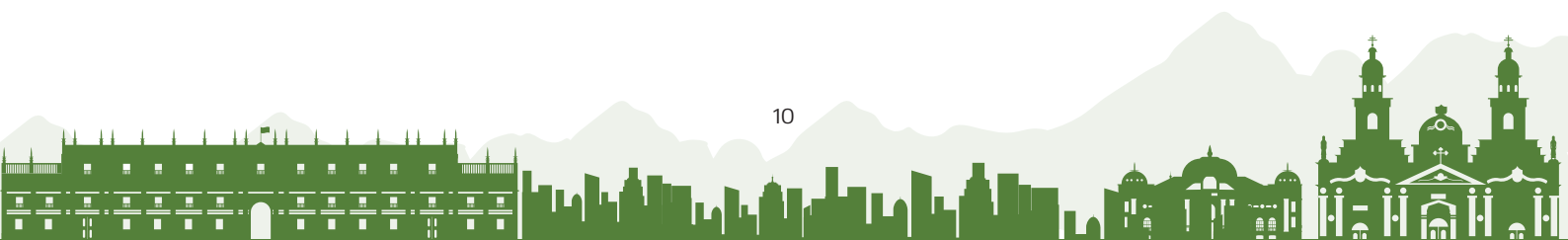
### OP-014

#### Complementary Medicine in the approach to complex patients in Clinical Veterinary Medicine and Comparative Medicine: Traditional Chinese Medicine & Apitherapy integrated into the Clinic. Clinical cases - animals; introduction to the subject - people

María Alejandra López Pazos

Terapis (R)

The development of Treatment Protocols that include Complementary Medicines in the clinical management of patients from different animal species, has been possible thanks to the study with scientific evidence of the properties of the Beehive products and their Synergy; using Traditional Chinese Medicine as a Medical System, together with Modern Medicine (Allopathic). The approach to patients through various specialties such as Internal Medicine, Surgery, Emergency Medicine; and combining different therapeutic tools (Apitherapy, Phytotherapy, Nutritional Support, Physiotherapy); to treat and support many animal species; has provided efficient alternatives to patients with very complex pathologies. I highlight Apitherapy in the therapeutic results of these clinical cases that are presented. To obtain the best results, we must pay attention to the quality of bee products and the specific protocol for the needs of each patient. To achieve this, in the practical work we include the supply of the best quality beehive products; in Nutraceutical formats for oral use, topical, surgical and injectable formulations. This Integrative approach of medical systems and techniques has allowed complex patients to walk from imbalance or pathology towards the balance of health. The presentation graphically shows examples of Integrative Treatments, including bee products in different animal patients and applied by different routes of administration. To simplify the presentation, the patients were ordered by medical specialties: Neurological / neuromuscular diseases, Degenerative diseases, Autoimmune diseases, Oncology. Reference is made to Comparative Medicine in people with similar pathologies and results.





## Apitherapy

### OP-015

## The Potential of Texan Honey to Treat and Prevent Nosocomial Infections

Ferhat Ozturk

Department of Integrative Biology, University of Texas San Antonio, San Antonio, TX, USA

Honey has been used for thousands of years as a powerful therapeutic agent due to its bioactivity potential, including antimicrobial, antioxidant, and anti-inflammatory properties, making it effective for wound healing. Despite the growing trend in biomedical research and clinical trials on the medicinal use of honey, little is known about the bioactivity potential and medical grade of the honey produced in the United States, including Texas.

To fill this knowledge gap, we collected a total of 110 honey samples from beekeepers in various regions of Texas, with the majority originating from San Antonio, between 2020 and 2022. We analyzed the samples for their conformity, antimicrobial and antioxidant properties using the following assays:

Conformity: Color intensity, pH, moisture, and pollen

Antimicrobial activity: Zone of inhibition, XTT, and H<sub>2</sub>O<sub>2</sub> assays

Antioxidant activity: TPC, TFC, DPPH, and FRAP assays.

Our results show that some of the honey samples analyzed in this study demonstrated high antimicrobial and antioxidant activities, indicating the potential for medicinal and veterinary use. Our study provides new and valuable insights into the bioactivity potential of Texas honey, which has not been thoroughly evaluated for its medicinal properties. Moreover, these results suggest that Texas honey could be considered as medical grade, offering a novel alternative to the current honey-based wound-healing agents in the market.

Our next step is to conduct clinical trials on patients with different wounds and monitor them throughout the healing process, using the highest potential honey samples from this study.

### OP-016

## Antinociceptive and Anti-inflammatory Effects of Bee Venom Injections on Osteoarthritis in Canines: A Pilot Test

Jorge Alberto Corredor Díaz

Department of Veterinary Medicine, University de los Llanos, Amazon, Colombia

Osteoarthritis (OA), also known as degenerative joint disease, is a chronic inflammatory disease that causes pain, stiffness, swelling and lameness due to the diminished cushion and changes in the synovial fluid.

Bee venom (BV) is used for long-term treatment of inflammation, pain and arthritis. Several compounds are purified from BV, such as melittin, adolopin, apamin, phospholipase, and amines. They seem to be responsible for the relief of these symptoms.

During a period of 14 months, 54 canines were evaluated by three experienced veterinarians using clinical methods, including goniometry and x-ray examination to verify diagnosis. All the animals had earlier been treated with a variety of therapies and pain killers with no satisfactory results.

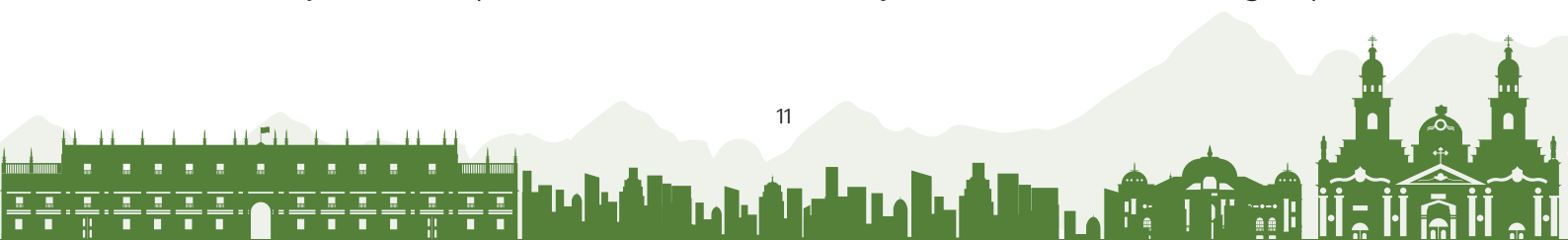
Therapy with bee venom consisted of an eight-week protocol of injections of three (3) international units (IU) of diluted Apitoxin injections introduced at seven different accupoints (via subcutaneous). Subjects received one treatment per week for eight weeks.

Thirty-five canines met the criteria to participate in the pilot trial. Pain relief was measured at Day 0, Day 15, and Day 35. There was a significant change in pain relief in 33 of the animals.

The Canine Brief Pain Inventory (CBPI) was the pain assessment model chosen for this study. Dogs received bee venom therapy on Day 1 and every week thereafter for a total of eight weeks.

Significant differences were detected in median pain scores for pain severity and pain interference (7.14 and 4.23 on Day 1 and Day 15, respectively). A significant improvement in pain severity and pain interference was observed in Week 5 (Day 35), where a decrease in the median scores was observed.

It seems that bee venom harnesses the power of a potent anti-inflammatory via IL-10-producing Tr1 cells to normalize glial activity and relief OA. Our results indicated that CBPI scores improved from weeks 2 to 5 in 94.29 % of the dogs included in this study. Our results provide novel evidence that BV injections are critical for treating OA pain.



## Apitherapy

### OP-017

#### Novel therapy with Colombia red propolis to treat feline leukemia. A remission of symptoms

Jorge Alberto Corredor Díaz

Department of Veterinary Medicine, University de los Llanos, Amazon, Colombia

Feline leukemia virus (FeLV) is one of the most common infectious diseases in cats, affecting between 4 to 6 percent of felines in Colombia. Infection rates are significantly higher (up to 30%) in cats that are ill or otherwise at high risk.

Viral particles are made up of the same components found in the structures of other living beings, this makes viral diseases difficult to control, because viruses can invade cells and damage their metabolism.

Propolis flavonoids induce the production of Interferons (INFs). These substances have several antiviral effects, including strengthening the cell membrane.

Propolis and its derivatives can inhibit the spread of viruses. Several studies have shown the effect of propolis on the RNA and DNA of various types of viruses, including Herpes type 1, Herpes type 2 and Adenovirus type 2.

Indirect immunofluorescent antibody assay (IFA) test was used after a positive ELISA test to confirm FeLV infection. FeLV vaccines will not cause false positive FeLV results on ELISA, IFA, or any other available FeLV tests.

This study of clinical cases in the city of Bogotá lasted four years and was carried out in 4 different clinics in the city with the help of other three veterinarians.

In this study 45 infected cats were diagnosed with FeLV and treated orally with Colombia red propolis tincture (30% concentration) for eight weeks. 42 (93.3%) of these cats showed complete remission of their clinical symptoms for at least 8 months.

A larger number of patients is needed to ensure that this treatment is effective in the remission of the symptoms of this contagious and deadly disease.

### OP-018

#### Apitherapy as an integrated approach to the support of autistic children

Natalia Sorokova

The health club "Apiculture". Russia, Moscow

The problems of children with autism pathology as well as with developmental delays have gone far beyond the problems of the parents of these children.

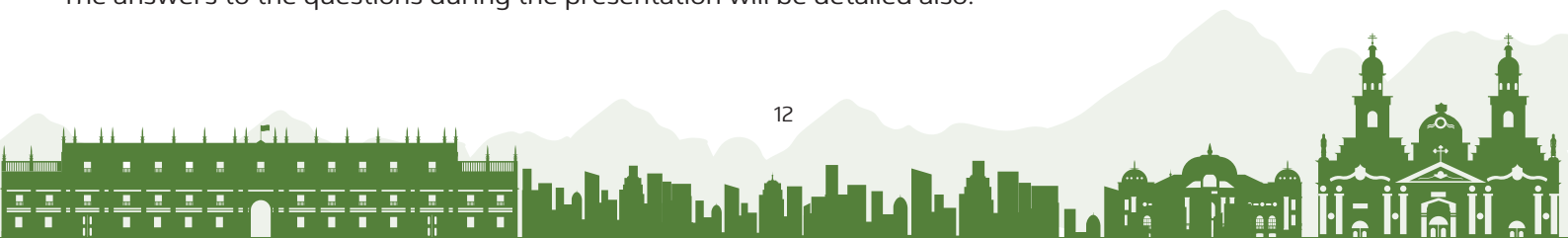
Experts around the world recognize the high development potential of children with autism pathology, calling them as special children with special abilities. Therefore, when choosing methods of working with these children, it is necessary to take into account the integrity of such a method in relation to the nature of the pathology itself. The method itself should imply and carry a healing effect. Apitoxin therapy is a progressive method that involves the regulation of the physiological processes.

Apitoxin has a regulatory effect on organs and organ systems. And also this drug has an effect on the adrenal cortex. Besides it has an effect on the central, immune and peripheral nervous system. In addition, it affects the central, immune and peripheral nervous system, participates in the production of endorphins, promotes the maturation of nerve cells, strengthens interneuron connections and regulates cerebral blood flow. Thus, it improves the process of learning to coordinate movement.

The child progresses with each session of apitoxin therapy, indicators of psychological and pedagogical support increase.

There will be three Clinical Cases concerning children with autism pathology. These cases are supposed to be described in detail and discussed during the presentation. Effect and acquired skills are to be explained.

The answers to the questions during the presentation will be detailed also.



## The Search for Medicinal Properties for Human Health of Spotted Lantern Fly Honey

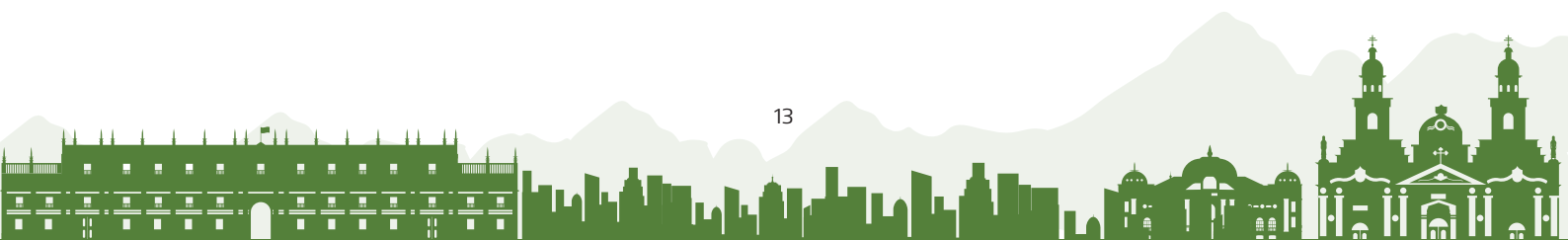
Petrusia Kotlar<sup>1</sup>, Robyn Underwood<sup>2</sup>, Ferhat Ozturk<sup>3</sup>

<sup>1</sup>American Apitherapy Society

<sup>2</sup>Department of Entomology, The Pennsylvania State University

<sup>3</sup>Department of Integrative Biology, University of Texas San Antonio

The Spotted Lanternfly (SLF), *Lycorma delicatula*, a beautiful plant hopper, recently introduced invasive species to the northeastern United States, is affecting apiculture by altering typical honey production by *Apis mellifera*. SLF are phloem feeders, puncturing the stems and trunks of over 170 plant species. They excrete large quantities of sticky, sweet honeydew as they feed. Honey bees convert the honeydew into a unique type of honey. Honey made from SLF honeydew has a smoky odor, the color is dark brown but not as dark as buckwheat honey, is not as sweet as other honey and has a lingering aftertaste. In general, honeydew honeys have more minerals, and macro/micronutrient than floral honeys. We aim to test this unique honey to determine whether the unique characteristics of SLF honey have potential apitherapeutic benefits for human health. Standard indicators such as diastase, proline, acidity, antioxidant activity, and phenolic and mineral compounds will be tested to determine the human health benefits of SLF honey. Previous work has shown that this honey contains SLF DNA and a compound, ailanthone, made by host plant Tree of Heaven, *Ailanthus altissima*. Ailanthone is a novel and potential drug for treating human cancer. Similar to the Unique Manuka Factor (UMF), a grading system developed by the UMF Honey Association in New Zealand for Manuka honey from the *Leptospermum scoparium* species, SLF honey sourced from the Tree of Heaven, *Ailanthus altissima* producing ailanthone could be the new "Unique Factor" to be tested for in honey produced in the United States. Besides all the negatives regarding the SLF infestation in the northeastern USA, the honeydew honey supports healthy, strong colonies with ample overwintering food stores.



Dr. Adrián Marichal<sup>1</sup>, Dr. Adrián Marichal<sup>2</sup>, Dr. Adrián Ramos<sup>1</sup>, Dr. Adrián Ramos<sup>2</sup>, Lic. Claudia Rodriguez<sup>3</sup>,  
Enfermera Carla Burguez<sup>4</sup>, Nicolás Cardozo<sup>5</sup>

<sup>1</sup>Medicina Familiar y Comunitaria, Universidad de Montevideo

<sup>2</sup>UAI-AIACH, Universidad Abierta Interamericana, Buenos Aires

<sup>3</sup>Universidad de la República Oriental del Uruguay

<sup>4</sup>SOCUHE, Uruguay

<sup>5</sup>Laboratorio Apiter SA

### Introduction

In the understanding that in our country we have the raw material and the industry specialized in the processing and elaboration of products derived from Propolis, based on evidence published in scientific articles about the anti-inflammatory activity of propolis which is linked to the capacity of its constituent polyphenols (flavonoids and aromatic acids) to inhibit NF- $\kappa$ B (nuclear transcription factor kappa-b) and reducing the levels of inflammatory mediators with a decrease in inflammation, pain and microbial charge. Therefore, it was decided to study the clinical efficacy of this product in complex wounds by means of a descriptive cross-sectional study.

### Objectives

To demonstrate the efficacy of this active ingredient in the control of bacterial load, immunomodulation, pain and wound healing.

### Materials

Materials for healing, products for moist wound care with Propolis in its dressing, lotion and ointment presentations

### Methods

The method to be used is the observation and assessment of users, data collection from electronic medical records, individualized assessment instruments for each wound, wound measurements (MOWA, ACETATE) and VAS as a pain scale.

### Results

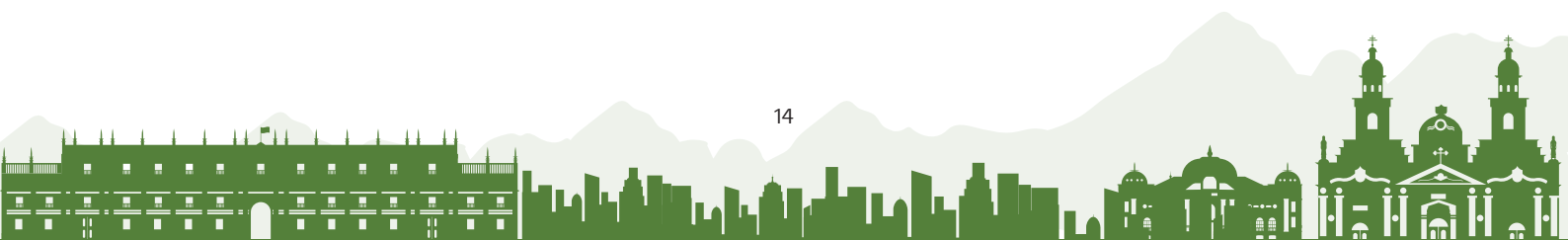
The study started on 2/1/2019, cut-off on 18/6/19.

We currently have 32 patients under study with the following characteristics and RESULTS: Of the total number of chronic wounds treated 60.5 % were venous ulcers, 12.5 % pressure ulcers, 12.5 % diabetic foot ulcers. 54 % were women, 56 % were over 60 years. 84 % reduced 2.5 cm<sup>2</sup> weekly.

### Conclusion.

So far we can say that the efficacy of propolis products can be clinically proven to reduce pain, inflammation and, as mentioned above, to promote healing in a moist environment. It is essential to emphasize that in order to carry out a scientifically rigorous study we must have adequate bacteriological studies (punch and/or percutaneous aspirate, electronic microscopy), and we consider that these techniques should be incorporated if we continue to do so.

We also conclude that the number of patients and the study time are limited, which is why it must be continued.



### OP-021

## Structural damage of a Mexican propolis on *Candida* species from clinical isolates by fluorescence microscopy

Betsabé Rodríguez<sup>1</sup>, Francisco Rodolfo González<sup>2</sup>, Carlos Gerardo García<sup>3</sup>, Tonatiuh Alejandro Cruz<sup>1</sup>

<sup>1</sup>Laboratorio del Servicio de Análisis de Propóleos (LASAP), Universidad Nacional Autónoma de México, Estado de México, México

<sup>2</sup>Laboratorio del Patología molecular veterinaria laboratorio A, Universidad Nacional Autónoma de México, Estado de México, México

<sup>3</sup>Laboratorio de Morfología Veterinaria y Biología Celular, Universidad Nacional Autónoma de México, Estado de México, México

The increase in resistance to antifungals and the side effects they cause in patients with infections caused by different species of "*Candida*", has led to considering treatments with natural products, such as propolis, which is a complex mixture collected by bees from the surrounding vegetation and which has shown antifungal activity due to its content of phenolic compounds and flavonoids. **OBJECTIVE:** To evaluate the structural damage of a Mexican propolis on different species of *Candida* from clinical isolates by means of fluorescence microscopy. **METHODS:** A propolis from the state of Morelos, Mexico was used. Phenolic and flavonoid compounds were quantified and the compounds present were identified by chromatography techniques. Antimicrobial activity was evaluated by the agar diffusion method, plate microdilution, and cell damage by fluorescence microscopy against clinical strains of "*Candida albicans*", "*Candida lusitanae*" and "*Candida tropicalis*". **RESULTS:** The concentration of phenolic compounds was  $2040 \pm 0.02$  eq AG/g propolis (20.4%) and  $1510 \pm 0.14$  eq Q/g propolis of flavonoids (15.1%), predominantly flavonoids, terpenes and fatty acids. The minimum inhibitory concentration (MIC) for "*C. albicans*" was 0.3 mg/mL, for "*C. lusitanae*" and "*C. tropicalis*" 2.5 mg/mL, and a minimum fungicidal concentration (MFC) of 1.25 mg/mL for "*C. albicans*" and 5 mg/mL for "*C. lusitanae*" and "*C. tropicalis*". Fluorescence microscopy showed that there is structural damage after 24 h, remaining until 48 h, although for "*C. albicans*" and "*C. tropicalis*" it decreased at 48 h, while for "*C. lusitanae*" it was maintained, so it was more effective against the latter species. **conclusions.** The antifungal activity of a Mexican propolis was demonstrated by the structural damage of "*C. albicans*", "*C. lusitanae*" and "*C. tropicalis*" through fluorescence microscopy.

### OP-022

## Susceptibility of microorganisms from clinical and reference isolates to Mexican propolis from native stingless bees

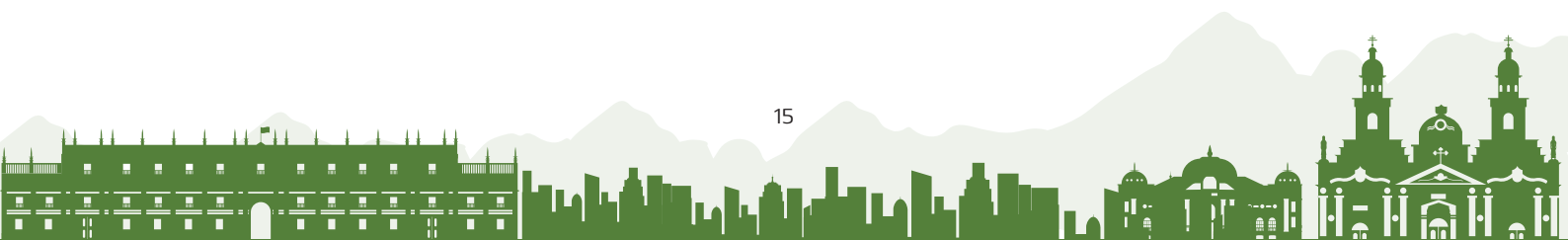
Betsabé Rodríguez<sup>1</sup>, Florentina Hernández<sup>2</sup>, Omar Rodrigo Guadarrama Escobar<sup>3</sup>, Tonatiuh Alejandro Cruz<sup>1</sup>

<sup>1</sup>Laboratorio de Servicios de Análisis de Propóleos (LASAP), Universidad Nacional Autónoma de México, Estado de México, México.

<sup>2</sup>El Colegio de la Frontera Sur (ECOSUR). San Cristóbal de las Casas, Chiapas, México.

<sup>3</sup>Laboratorio de sistemas transdérmicos y nanoestructurados, Universidad Nacional Autónoma de México, Estado de México, México.

Propolis from native stingless bees has a complex chemical composition, including phenolic compounds and flavonoids that confer antimicrobial activity. Therefore, 17 propolis from seven species ("*Friesiometitta nigra*", "*Melipona beecheii*", "*Nannotrigona perilampoides*", "*Plebeia frontalis*", "*Plebeia*" sp, "*Scaptotrigona pectoralis*" and "*Tetragonisca angustula*") were collected in the states of Chiapas and Veracruz (Mexico) to determine their concentration of phenols and flavonoids, as well as their antimicrobial activity, as well as their antimicrobial activity (antibacterial and antifungal) against reference strains and clinical isolates of "*Staphylococcus aureus*", "*Escherichia coli*", "*Klebsiella pneumoniae*", "*Pseudomonas aeruginosa*" and "*Candida albicans*". The results showed statistically significant differences ( $p < 0.05$ ) in the amount of phenolic compounds ranging from 244 to 2482 mg AG/g propolis (mg gallic acid equivalents) and also in flavonoids from 91 to 690 mg Q/g propolis (mg quercetin equivalents), as well as in antimicrobial activity, where it was shown that the minimum bactericidal concentrations were between 0.26 and 21.33 mg/mL and the minimum fungicidal concentrations for "*Candida albicans*" were between 2.37 and 23.11 mg/mL. The best antimicrobial activity was presented in propolis of "*Plebeia frontalis*", "*Scaptotrigona mexicana*", "*Friesiometitta nigra*" and "*Nannotrigona perilampoides*" species. The results highlight the biological potential of native stingless bee propolis for its possible application as an alternative agent in the treatment of "*Staphylococcus aureus*", "*Escherichia coli*", "*Klebsiella pneumoniae*", "*Pseudomonas aeruginosa*" and "*Candida albicans*" infections.



## Apitherapy

### OP-023

## Propolis and Honey-Copper Nanoparticle Formulation: An Effective Approach for Enhancing Antibacterial Activity in Chronic Wound Healing

Limberg Jaldin-Crespo, Fabian Zuñiga, Marcelo Ezquer, Jessica Isabel Martinez Arenas

Center for Regenerative Medicine, Faculty of Medicine Clínica Alemana-Universidad del Desarrollo, Santiago-Chile.

Chronic wounds are a significant healthcare challenge, often complicated by the presence of antibiotic-resistant bacteria. This study investigated the potential of propolis, and honeycopper nanoparticles (HoneyCuNP) as a therapy for chronic wound healing. Here we characterized the nanoparticles using transmission electron microscopy (TEM), dynamic light scattering (DLS), and polydispersity index (PDI). The size of the nanoparticles was found to be  $2.7 \pm 0.6$  nm, and they exhibited a spherical shape, as determined by TEM. The  $\zeta$ -potential measurements revealed that the nanoparticles were stable and had a potential of  $-24,8 \pm 4,9$  mV. A PDI value of  $0.4 \pm 0.02$  indicates a moderate degree of size distribution in a nanoparticle formulation. Antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, was assessed using standard microbiological methods. Here we found that the combination of propolis, and HoneyCuNP in the formulation demonstrated an enhancing effect, significantly inhibiting bacterial growth compared to the individual components alone. This study highlights the potential of natural agents and nanotechnology for developing effective therapies for chronic wound healing, specifically against the bacteria most prevalent in chronic wounds.

In conclusion, the propolis and HoneyCuNP formulation with well-characterized nanoparticles and a stable  $\zeta$ -potential holds great promise as an effective therapy for chronic wound healing, offering a promising alternative to traditional antibiotics.

### OP-024

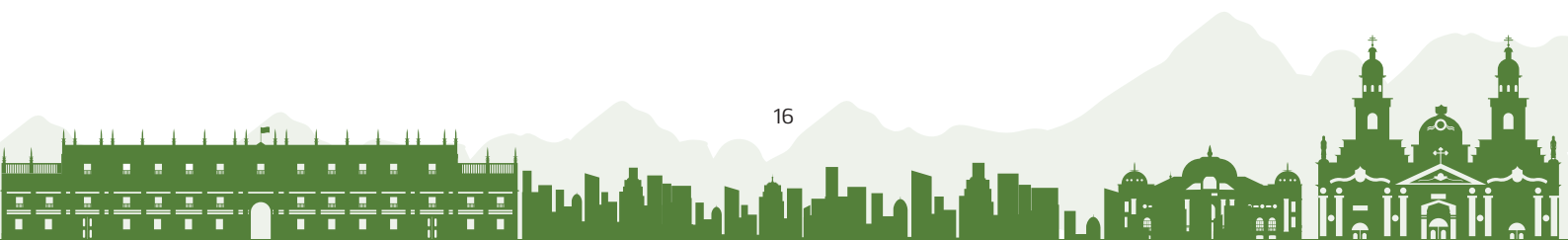
## Improvement of the antimicrobial activity of Mexican propolis with synthetic chalcones

Betsabé Rodríguez<sup>1</sup>, Jose Guillermo Penierres<sup>2</sup>, Tonatiuh Alejandro Cruz<sup>1</sup>

<sup>1</sup>Laboratorio del Servicio de Análisis de Propóleos (LASAP), Universidad Nacional Autónoma de México, Estado de México, México

<sup>2</sup>Laboratorio de Química Verde, Química Orgánica, Universidad Nacional Autónoma de México, Estado de México, México

Introduction. The antibacterial activity of propolis is related to the content of phenolic compounds and flavonoids; however, not all propolis present this property due to the absence or low amount of these compounds, which can be increased with the addition of chalcones. Chalcones are compounds distributed in nature and are biosynthetic precursors of flavonoids. Their chemical structure presents an aromatic ring at each end of a chain of three carbon atoms with an  $\alpha, \beta$ -unsaturated carbonyl system, where the rings can be substituted or not by hydroxyl or methoxyl groups, regularly. Authors report the relationship of biological activities with the mentioned structural features or with modifications of them. Objectives. To synthesize three chalcones to potentiate "in vitro" antibacterial activity by agar diffusion method against reference strains of "*Staphylococcus aureus*" and "*Escherichia coli*". Material and methods. Three chalcones were synthesized in the laboratory by Claisen-Schmidt condensation: 1,3-diphenylpropyl-2E-1-one (PLCR-1) and two analogues with hydroxyl substituents: 1-(3,4-dihydroxyphenyl)-4-(2,4,6-trihydroxyphenyl)prop-2E-en-1-one (PLCR-2) and 1-(3,4-dihydroxyphenyl)-4-(2,4-dihydroxyphenyl)prop-2E-en-1-one (PLCR-3) which were added in different proportions, to two propolis, one from the State of Guanajuato (low amount of flavonoids) and the other from the State of Mexico (with flavonoids) to evaluate the antibacterial activity against reference strains of "*Staphylococcus aureus*" and "*Escherichia coli*" using the disk diffusion method. Results. Only chalcone PLCR-3 enhanced the antibacterial effect of both samples against "*S. aureus*". The increase in the antibacterial activity of a propolis with the addition of synthetic chalcones is related to the microorganism evaluated and the proportion used.





## Effect of *Melipona beecheii* pot-pollen proteins on "*Pseudomonas aeruginosa*" biofilm formation

Elizabeth Ortiz-Vázquez<sup>1</sup>, Harumi Caroline Sansores Caamal<sup>1</sup>, Jesus Manuel Ramon Sierra<sup>1</sup>, Radamés Trejo Valencia<sup>2</sup>, Alejandro Yam Puc<sup>1</sup>

<sup>1</sup>División de Estudios de Posgrado e Investigación, Tecnológico Nacional de México/ITMérida, Mérida, México

<sup>2</sup>Departamento de Ingeniería Química Bioquímica, Tecnológico Nacional de México/ITMérida, Mérida, México

Pot-pollen from stingless bees like *Melipona beecheii* has antimicrobial properties conferred by biomolecules in this natural product. The wide variety of phenolic and protein compounds in pot-pollen come from the plants that bees forage. Yucatan has plants such as: *Gymnopodium floribundum*, *Bursera simaruba*, *Passiflora foetida* L., among others, which have been used for their medicinal properties in Mayan culture. *M. beecheii* is very careful in the selection of plants that it uses to collect pot-pollen or bee bread, which is defined as fermentation product from pollen plants collected by bees, also they added salivary enzymes and honey. The main goal of this study was to evaluate the antimicrobial and antibiofilm activities of proteins obtained from *M. beecheii* pot-pollen against *Pseudomonas aeruginosa* ATCC 27853.

Fifteen sharp bands (proteins) were observed in an electrophoretic analysis (SDS-PAGE) performed on *M. beecheii* Pot-Pollen Proteins (MPPP). Those proteins were responsible of the biological activity, since the whole crude protein extract had antimicrobial and antibiofilm activity against *P. aeruginosa* using 1.7 µg/mL of protein extract. On the other hand, MPPP extract caused damages in the morphology of *P. aeruginosa*, which were observed through Scanning Electron Microscopy (SEM). The *fleN*, *fleQ* and *fleR* gene expression of *P. aeruginosa* were determined by RT-PCR, MPPP extract decreased the expression levels of those genes. More, changes in secreted proteins were observed during growth of *P. aeruginosa* in Trypticase Soy Broth added with MPPP.

Prevention and adequate treatments to *P. aeruginosa* are essential because this bacterium is a nosocomial hosted. This microorganism mainly attacks immunocompromised patients, people in intensive care and newborns, causing high rates of morbidity and mortality. The World Health Organization (WHO) reports that one in every ten affected patients for nosocomial diseases will be die. *P. aeruginosa* is classified in the critical category due to its multiple virulence factors and resistance mechanisms.

This research demonstrated the scope that MPPP possesses and the potential use of proteins from *M. beecheii* pot-pollen for treating bacterial infections caused by *P. aeruginosa*, so that could serve as alternative therapy.



### OP-026

## Unravelling the beehive air volatiles profile as analysed via solid-phase micro-extraction (SPME) and chemometrics and its role in combating Asthma

Hesham El-Seedi<sup>1</sup>

<sup>1</sup>Pharmacognosy Group, Department of Pharmaceutical Biosciences, Uppsala University, Biomedical Centre, Box 591, SE 751 24 Uppsala, Sweden

<sup>2</sup>International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang 212013, China

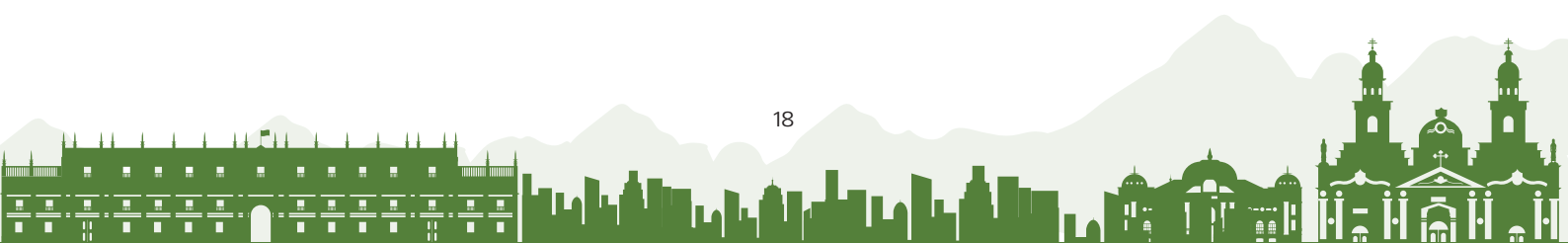
The honeybees (*Apis mellifera* L.) have several products, including honey, propolis, royal jelly, bee venom, and bee pollen. Bee products meet the criteria of being natural products that have long-recognized medicinal properties. Historically, bee products nutritional and medicinal values have been considered for thousands of years by Ancient Egyptian, Persians, Romans, and Chinese in supplementary nutrition and alternative diets<sup>1,2</sup>. Recently, beehive air therapy is recognized as a potential remedy for treating asthma, bronchitis, lung fibrosis, and respiratory tract infections. Developed countries in which beehive air therapy is currently authorized include Germany, Hungary, Slovenia, and Austria. However, scientific proof of its efficacy is lacking which warrants further chemical and biological analyses as a proof of concept. In this study, beehive air volatile profile was determined for the first time along with its individual components.

Volatile compounds were collected from beehive air using solid phase micro-extraction (SPME) coupled to gas chromatography-mass spectrometry (GC-MS). Antimicrobial assay was performed against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and multi drug-resistant *Staphylococcus aureus* (MRSA) using the in vitro agar-well diffusion and microtiter plate assays<sup>3</sup>.

A total of 56 volatile compounds were identified from beehive air, venom, bee insect and wax air including fatty acids, alcohols, aldehydes, esters, ether, hydrocarbons, phenol, ketones, nitrogenous compound, and terpenes. The most abundant constituents were short-chain fatty acids. The principal component analysis (PCA) scores plot of the UPLC/MS dataset showed the similarity of the beehive air to the insect bee's aroma profile. Beehive air and venom exerted the strongest antimicrobial activity.

### References

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2. El-Seedi HR, et al., Bee pollen: Current status and therapeutic potential. *Nutrients*. 2021 May 31;13(6):1876.
3. El-Seedi HR, et al., Unravelling The Beehive Air Volatiles Profile As Analysed Via Solid-Phase Microextraction (SPME) And Chemometrics. *Journal of King Saud University-Science*, 2021, 33, 101449.





### OP-027

## Synergistic Effect of Melipona Honey with Conventional Antibiotics against E. coli ATCC 25922

Elizabeth Ortiz-Vázquez<sup>1</sup>, Hajar Ennya<sup>1</sup>, Jesus Manuel Ramon Sierra<sup>1</sup>, Enrique Peraza-López<sup>1</sup>, Oscar May Tzuc<sup>2</sup>, Ali Bassam<sup>3</sup>

<sup>1</sup>División de Estudios de Posgrado e Investigación, Tecnológico Nacional de México/ITMérida, Mérida, México

<sup>2</sup>Departamento de Posgrado, Universidad Autónoma de Campeche, Campeche, México

<sup>3</sup>Facultad de Ingeniería, Universidad Autónoma de Yucatán, Mérida, México

Melipona beecheii is an endemic stingless bee from the Yucatan Peninsula, Mexico. The use of this species dates from prehispanic era, its honey has been used as a traditional remedy for different dermal, digestive and respiratory diseases. It has been demonstrated in vitro that the M. beecheii honey has antimicrobial activity against gram positive, gram negative and opportunistic yeasts such as Candida albicans. The increase of resistant microorganisms to commonly used antibiotics, has motivated the search for new alternatives to combat or assist in the inhibition of these pathogens. In the present work, we evaluated the effect of bacterial growth using M. beecheii honey with each of three conventional antibiotics (amoxicillin, ciprofloxacin and amikacin). Our goal was to find the synergistic effect of M. beecheii honey against Escherichia coli ATCC 25922. M. beecheii honey was collected in Mani, Yucatán directly from the hive. Microdilution techniques were carried out to determine the Minimum Inhibitory Concentration (MIC) for each one; three antibiotics and M. beecheii honey. Subsequently, MIC of M. beecheii honey added with each of the antibiotics was determined. The results were analyzed through an artificial intelligence model using a genetic programming model integrated into a numerical computing system (MATLAB). The MIC of M. beecheii honey was 13% (w/v), while the MIC of amoxicillin, ciprofloxacin and amikacin were 0.08 µg/mL, 0.007 µg/mL and 2 µg/mL respectively. When the honey was added to each antibiotic, the MIC was 9, 10 and 20 times less for amoxicillin, ciprofloxacin and amikacin respectively. Even the honey MIC decreased 1.6 times. The MATLAB analysis demonstrated that M. beecheii honey had a synergistic 8% potentiating effect.

The M. beecheii honey presented synergistic effect with each of the antibiotics evaluated in this study. Therefore, this honey presents an opportunity as an adjuvant against the treatment of diseases related to this bacterium, it would have to be validated in vivo.

### OP-028

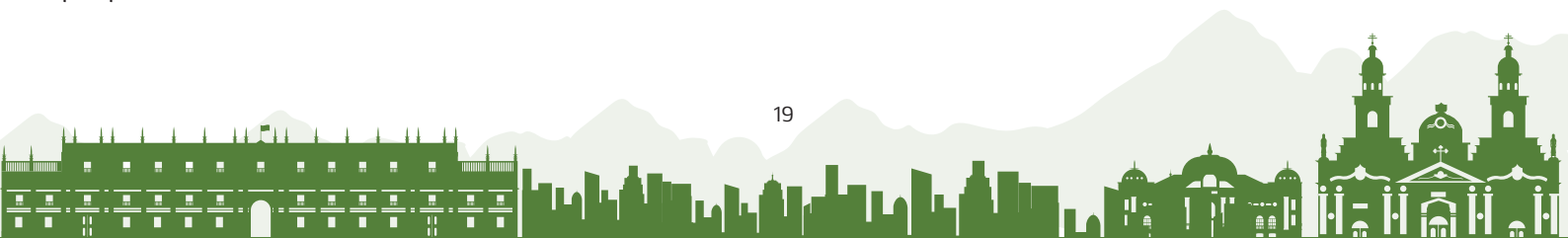
## Transdermal patches loaded with polymeric nanoparticles of propolis as a wound healing enhancer in diabetics

Omar Rodrigo Guadarrama Escobar<sup>1</sup>, Betsabé Rodríguez<sup>2</sup>, Nayeli Ruiz Castañares<sup>2</sup>, Lizeth Andrea Esteban Ochoa<sup>2</sup>, Tonatiuh Alejandro Cruz<sup>2</sup>, José Juan Escobar Cháve<sup>21</sup>

<sup>1</sup>Unidad de Investigación Multidisciplinaria-Lab 12, Facultad de Estudios Superiores Cuautitlán-Universidad Nacional Autónoma de México, Carretera Cuautitlán-Teoloyucan, km 2.5 San Sebastián Xhala, Cuautitlán Izcalli 54714, Mexico

<sup>2</sup>Laboratorio de Servicio de Análisis de Propóleos (LASAP), Unidad de Investigación Multidisciplinaria (UIM), Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Cuautitlán Izcalli, 54714. Mexico

**INTRODUCTION:** Diabetes mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia that is accompanied, to a greater or lesser extent, by the alterations in carbohydrate metabolism. Diabetic foot ulcers are among the most common complications of patients who have diabetes mellitus which is not well controlled. Bee propolis is a natural substance that is used in traditional medicine due to its versatile pharmacological actions. The main objective is to develop and provide a new nanoparticle vehicle for topical propolis for wound treatment in people with diabetes mellitus. **METHODOLOGY:** The nanoparticles were made according to the methodology described by Guadarrama Escobar et. al., which were incorporated and used as a dispersing agent in the formulation of the patch. The transdermal patch was made according to the research carried out by Serrano Castañeda et. al., which consists of a bilayer patch, each with different properties. Nanoparticles as well as transdermal patches were characterized physicochemically to know their properties of resistance to rupture, bioadhesion and bioadhesion postwetting, to provide a suitable pharmaceutical form for the administration and treatment of superficial wounds. **RESULTS:** The patches showed to have adequate physical characteristics to be used as suitable transdermic systems for the administration of nanoparticles with propolis to promote healing. the results of the tests are then: breaking strength 2063±965 g/F, bioadhesion 1027.33±419g/F and postwetting 293.08±36g/F. **CONCLUSION:** Transdermal bilayer patches were formulated with the appropriate physical characteristics (rupture resistance, bioadhesion and postwetting) to be considered as a novel alternative for the treatment of skin wounds in people with diabetes.



## Apitherapy

### OP-029 Redefining Medicine

James Fearnley

Apiceutical Research Centre, UK

Modern pharmaceutical medicine is in crisis. The magic bullet is proving ever more elusive and inappropriate. The economically driven pharmaceutical juggernaut trundles on but the definition and meaning of medicine is being redefined – from targeted, synthetic, and single molecule to natural, synergistic, holistic, sustainable, ecological. Over 40 years ago the Kazan Veterinary Institute published research which showed that combining propolis with antibiotics increased the effectiveness of the antibiotics by up to 100 times. We had to wait till the 1990's before the West began to publish scientific papers. Research into propolis has grown exponentially over the last ten years with Brazil, China, Turkey and India now major contributors. Research in UK has grown over the last 15 years particularly at the University of Strathclyde - Scotland. They have developed our understanding of how the chemical compounds collected by the honey bee from plants and trees within their local environment are transformed by the honey bee into a product able to provide immune defence for the whole superorganism. The relationship between climate and the antibacterial properties of propolis is now more clearly understood. The discovery of anti-trypanosome chemicals in propolis in areas where there is sleeping sickness has opened up new research into Ecological or Geographic Medicine. Since 2011 ARC has been linking research activity round the world through a series of international conferences and more recently through the formation of IPRG (International Propolis Research Group) ARC's Global BeePharma project further explores the concept of Geographic Medicine i.e. the chemical and biological activity of local bee products to local disease patterns in humans.

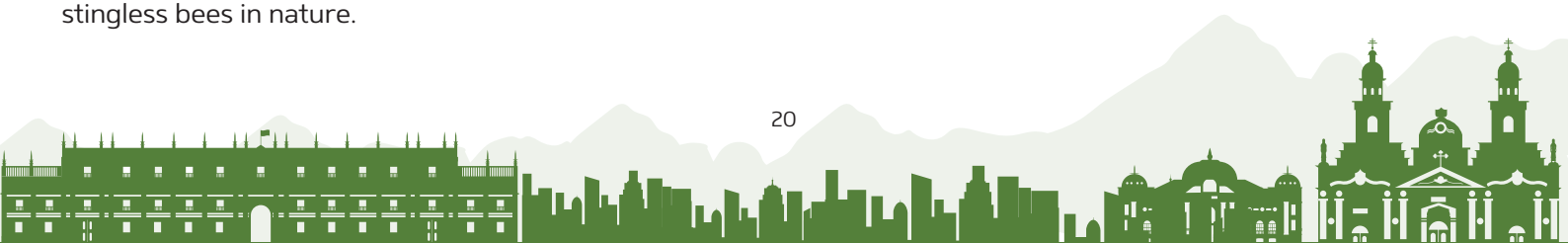
ARC has plans to build The BeeArc, a physical research centre and exhibition centre based in North Yorkshire UK and focussed on Apiceuticals – medicines from the beehive and Sustainable Beekeeping.

### OP-030 Diversity of active metabolites in stingless bee nest materials, a path for rationale discoveries in apitherapy

Patricia Vit

Apitherapy and Bioactivity, Food Science Department, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Merida, Venezuela

Stingless bees (Hymenoptera: Apidae: Apinae: Meliponini) collect biotic and abiotic materials from nature to be transformed into nest materials with diverse functions such as structural, immune, defense, food. A large number of 600 species of stingless bees collecting natural resources for their nests is a spectacular biodiversity, like the diversity of chemical structures in their nest materials, as evidenced in the book Stingless bee cerumen and propolis. The biotic materials processed to form pot-honey and pot-pollen have botanical, entomological, and microbial origins, as well as the plant resins needed for the vital cerumen in the stingless bee nest. A sequence of researches serving apitherapy illustrated the ancient use of pot-honey eye drops to the latest cerumen components and reducing oxidative stress led by a team at the Federal University of Grande Dourados in Brazil. Besides the chemical composition, the antioxidant activity is a fundamental added value for stingless bee nest materials, supporting a medicinal approach for both nutritional and pharmaceutical applications. The pot-honey spectra of aliphatic organic acids (AOA) show distinctive features among stingless bee genera associations with microbes. Increased AOA contents in fermented pot-honey is not a defect, but microbial associations with stingless bees preserving their wet honey with active metabolites. A Honey Authenticity Test by Interphase Emulsion (HATIE) became a Honey Biosurfactant Test (HBT) for visual detection of active metabolites such as putative sophorolipids produced by associated *Starmerella* yeast with stingless bees suspected to be present in the nest of Ecuadorian *Scaptotrigona vitorum* Engel 2022. Neotropical biodiversity of stingless bees was evidenced with a brief display of the 198 stingless bee species from Ecuador, a country 30 times smaller than Brazil with 244 stingless bee species. Active metabolites from the stingless bee nest are not envisaged to be extracted but to be used in their original matrices pot-honey, pot-pollen or cerumen. A synthesis of most active metabolites could be an option for pharmaceutical developments to reproduce a bioactive chemical repertoire of stingless bees in nature.



## Use of Propolis Alginate for the Treatment of Difficult Healing Wounds

Dr. Adrián Marichal<sup>1</sup>, Dr. Adrián Marichal<sup>2</sup>, Enfermera Carla Burguez<sup>3</sup>, Nicolás Cardozo<sup>4</sup>

<sup>1</sup>Medicina Familiar y Comunitaria, Universidad de Montevideo

<sup>2</sup>UAI-AIACH, Universidad Abierta Interamericana, Buenos Aires

<sup>3</sup>SOCUHE, Uruguay

<sup>4</sup>Laboratorio Apiter SA

On the production of propolis-based products, based on the evidence published in scientific articles that refer to the anti-inflammatory activity of propolis which is linked to the ability of its constituent polyphenols (flavonoids and aromatic acids) to inhibit NF- $\kappa$ B (nuclear transcription factor kappa-b) and reducing the levels of inflammatory mediators. This results in a decrease in inflammation, pain and microbial charge, so it was decided to clinically test the efficacy of this product on complex wounds by conducting a descriptive, prospective, cross-sectional study.

### General Objective

To demonstrate the reduction of wound healing time through the application of a propolis-impregnated dressing that controls the bacterial charge, acts on immunomodulation and pain.

### SPECIFIC OBJECTIVES

- Application of propolis dressings on users with venous ulcers, pressure sores, diabetic foot, atypical ulcers and neoplasms.
- Follow-up and evolution of the wound (photography, measurement of the area, Imito Wound).
- Measurement of pain scale.

### STUDY

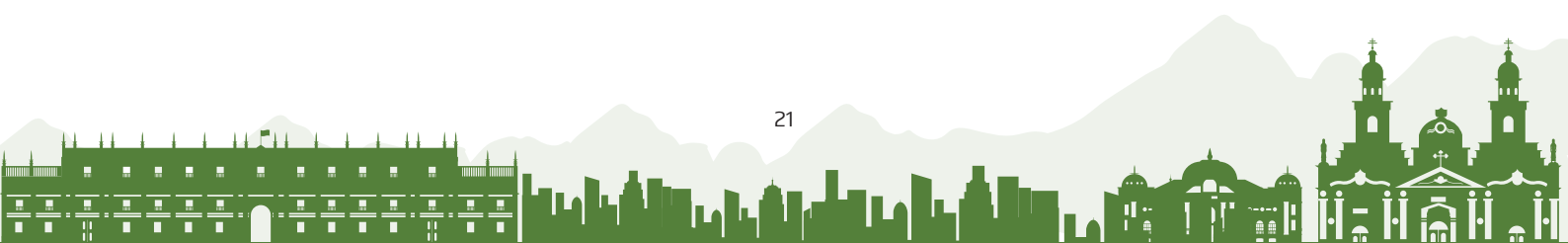
The study started on 2/3/20, a cut-off on 10/22. TOTAL: 31 MONTHS Total number of patients 40.

Comprised the following types of wounds:

- Venous Ulcers
- Arterial Ulcers or Non-Diabetes Arthropathies • Diabetic Foot
- Pressure Ulcers
- Mixed Ulcers
- Atypical Ulcers • Traumatic Ulcers • Malignant Ulcers

### Conclusions

So far we can say that the reduction of healing time is clinically verifiable, this means that in various types of wounds the benefits and qualities of propolis result in a reduction of inflammation, reduction of pain, control of the bacterial charge and therefore the wound bed heals in less time as well as providing an adequate moist environment in the wound.



### OP-032

## Honey bee populations of the USA reveal restrictions in their maternal genetic diversity

Mohamed Alburaki

USDA-ARS, Bee Research Lab, Beltsville, MD, USA

The genetic diversity of the USA's honey bee *Apis mellifera* L. populations was examined through a molecular approach using two mitochondrial DNA (mtDNA) markers. A total of 1,063 samples were analyzed for the mtDNA intergenic region located between the cytochrome c oxidase I & II (COI-COII) and 401 samples were investigated for the NADH dehydrogenase 2 (ND2) coding gene. The samples represented 45 states, the District of Colombia and two territories of the USA. Nationwide, three maternal evolutionary lineages were identified: the North Mediterranean lineage C (93.79%), the West Mediterranean lineage M (3.2%) and the African lineage A (3.01%). A total of 27 haplotypes were identified, 13 of them (95.11%) were already reported and 14 others (4.87%) were found to be novel haplotypes exclusive to the USA. The number of haplotypes per state/territory ranged between two and eight and the haplotype diversity  $H$  ranged between 0.236 - 0.763, with a nationwide haplotype diversity of 0.597. Furthermore, the honey bee populations of the USA were shown to rely heavily (76.64%) on two single haplotypes (C1=38.76%, C2j=37.62%) of the same lineage characterizing *A. m. ligustica* and *A. m. carnica* subspecies, respectively. Molecular-variance parsimony in COI-COII and ND2 confirmed this finding and underlined the central and ancestral position of C2d within the C lineage. Moreover, major haplotypes of *A. m. mellifera* (M3a, M7b, M7c) were recorded in six states (AL, AR, HI, MO, NM and WA). Four classic African haplotypes (A1e, A1v, A4, A4p) were also identified in nine states and Puerto Rico, with higher frequencies in southern states like LA, FL and TX. This data suggests the need to evaluate if a restricted mtDNA haplotype diversity in the US honey bee populations could have negative impacts on the beekeeping sustainability of this country.

### OP-033

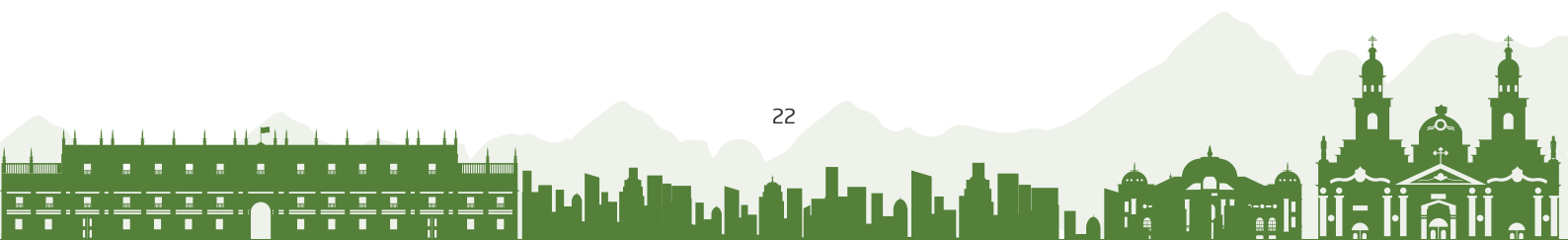
## Effect of essential oils on associative learning of honeybees

Guillermo Bragunde<sup>1</sup>, Andrea Carolina Sosa Labadie<sup>1</sup>, Andrés González<sup>2</sup>, Carmen Rossini<sup>2</sup>

<sup>1</sup>Laboratorio de Ecología Química, Facultad de Química, Universidad de la República, Uruguay; Graduate Program in Chemistry, Facultad de Química, Universidad de la República, Uruguay

<sup>2</sup>Laboratorio de Ecología Química, Facultad de Química, Universidad de la República, Uruguay

During their life cycle, honeybees are constantly exposed to xenobiotics from the environment and from in-hive products applied as pesticides. Many of these products do not produce honeybee mortality at the applied doses; however, their sublethal effects in physiological processes such as learning and memory are not often evaluated. Among potential new acaricides for honeybee colonies, plant essential oils (EO) have been found effective and safe for honeybees. However, EO intake can produce sublethal effects as has been shown for cuticular hydrocarbon profiles. To further characterize the potential chronic effects of EO exposure on associative learning, we exposed honeybees to the EO of *Eupatorium buniifolium* (Asteraceae) and to thymol, a natural in-hive varroacide, both in the laboratory and in-hive. Laboratory exposures were carried on cages with 200 honeybees. In the beehives, previously marked honeybees were retrieved after 12 days of treatment. In both cases treatments were applied on paper strips. The effects on associative learning were evaluated on 5-6 and 12-13-day-old honeybees for laboratory-treated honeybees, and on 13-17-day-old honeybees from treated hives. We used 1-hexanol as conditioned stimulus and sucrose 50% m/m as unconditioned stimulus to study associative learning using the Proboscis Extension Response (PER). The volatile stimulus was delivered using an automatic ad-hoc system. The rate of associative learning showed that exposure to the EO of *E. buniifolium* or thymol did not reduce the ability to associate a food reward with an odor, both in laboratory and in-hive exposure. These results suggest that the use of EO as acaricides may be safer than other products, and highlights the need for testing sublethal effects of potential sanitary products for honeybees.



### OP-034

#### Study of the reproductive performance of drones and queens bees of *Apis mellifera intermissa* in north of Algeria

Latrech Hamidou<sup>1</sup>, Belabdi Ibrahim<sup>1</sup>, Mimoun Nora<sup>2</sup>, Hamel Mouna<sup>1</sup>, Salma Roumissa<sup>1</sup>, Ait Gharbi Lakri<sup>1</sup>, Hammaz Malha<sup>1</sup>, Kaidi Rachid<sup>1</sup>

<sup>1</sup>Institute of veterinary Science, Biotechnology of Reproduction Laboratory, Blida, Algeria

<sup>2</sup>National higher veterinary School, Bab-Ezzouar, Alger, Algeria

Our studies were carried out in the region of Metidja, in the north of Algeria, from January 2016 to August 2019; the first part of the study consists in examining the production and the quality of the sperm of the drones from certain numbers parameters including age, weight and the season of rearing. The second part of the study was reserved for the grafting and the quality of the queens, the average weight of the queens at emergence and after mating, were evaluated, as well as the Spermatheca diameter and the number of spermatozoa in the queen's spermatheca, ovaries weight and ovarioles number. The morphological and biometric characters of queens and the correlations that may exist between these characters were also studied.

The results show that 34.45% of the drones examined released sperm by endophallus after manual eversion; the number of spermatozoa produced by drones is 5.750.000 and 3.380.000 spermatozoa in seminal vesical for Drones who do not produce semen during eversion. The spermatozoa number produce increases with weight, age and season of rearing.

The acceptance rate of larva grafted into queen right colonies was 75%, with 334 mg royal jelly produced by cup. The average weight of the queens at emergence and after mating were evaluated, as well as the number of spermatozoa in the queen's spermatheca. The results obtained show a high mortality rate of nymphs before hatching (26.66%), an average weight of virgin queens at emergence ( $0.160 \pm 0.011$ gr), and after mating is ( $0.176 \pm 0.017$ gr), a rate variable mating from 52.5% to 78.57% depending on the season, Semen analysis shows an average of 5,411,111  $\pm$  1915,361 spermatozoa in the queen's spermatheca after mating.

Concerning the study of the morphological and biometric characters of the queen, the results show that there is same Morphometrics correlations: Tomentum-Pilosity, Tomentum - Coloration, Tongue length-Cubital index. And same biometrics correlation: Queen weight-Spermatheca diameter, Queen weight- ovaries weight.

These studies underline the need to know more about the local bee, in order to characterize the local bees and to have a continuous improvement of production.

### OP-035

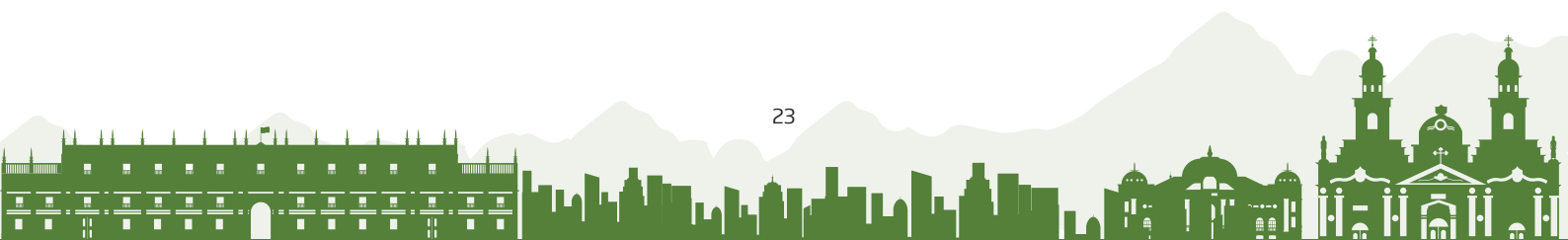
#### A semi-field method for measuring the impact of diet on brood production in intact honeybee nucleus colonies

Rui Fernando Da Silva Gonçalves<sup>1</sup>, Raquel Teixeira De Sousa<sup>1</sup>, Geraldine Wright<sup>1</sup>, Sharoni Shafir<sup>2</sup>

<sup>1</sup>John Krebs Field Station, Department of Biology, University of Oxford, OX2 8QJ Oxford, UK

<sup>2</sup>The Hebrew University of Jerusalem, The Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot 7610001, Israel

Honeybee populations in temperate climates fluctuate seasonally, boosting during summer and decreasing during winter. Colony nutrition is vital in population size because floral pollen and nectar largely determine how many broods a colony can rear. Studying which nutritional components of diet influence brood production in bee colonies is difficult, however, because it is hard to control what the whole colony eats, making it difficult to standardize experimental treatments across replicates. Here we describe a new method for measuring how diet influences brood production in honeybee colonies using styrofoam colony nucleus boxes (APIDEA®, Switzerland) maintained within a polytunnel. The polytunnel allowed us to control the environment of the colonies and limit or prevent food from entering the colony. Each colony consisted of a brood box, five small frames, a top feeder with a netted square hole for food delivery, one mated and laying queen, and 300–500 g of young workers. Food is delivered in a patty form inside, and the sugar solution is provided outside the nucleus box. We estimated the quantity of food consumed by nurse-age adult workers from each colony, the number of bee seams, nucleus weight, and the number of sugar stores over time. A photograph of each frame was also taken every 5-10 days over 25 days to assess the brood. We counted the number of eggs, larvae, and capped pupae using the count tool in Adobe Photoshop. Food efficiency can be calculated by dividing the total capped brood by the amount of food eaten over the assessment period. This method standardizes the nutrition of individual, intact honeybee colonies, enabling reproducibility and requiring fewer apiary resources. It will make it possible for researchers to measure the impact of nutrition on the performance of whole colony populations over periods as long as three months.





### OP-036

#### Determining the age at which worker honey bees interact with and consume pollen substitute patties

Emily Noordyke<sup>1</sup>, Kaylin Kleckner<sup>2</sup>, Cody Prouty<sup>2</sup>, James D. Ellis<sup>2</sup>

<sup>1</sup>Department of Primary Industries, New South Wales, Australia

<sup>2</sup>Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

Pollen is essential for honey bee brood development, but the quality and quantity of pollen in a given environment varies seasonally. Beekeepers often provide pollen substitute patties (PSPs) as a source of protein and micronutrients when high-quality natural pollen is unavailable in the environment. Our previous dye-tracing research has established that bees do not utilize PSPs the same way they utilize natural pollen. While a significant portion of adult workers consumed PSPs in our studies, they did not store it as bee bread, neither did nurse bees (adult workers 3-15 days old) feed the PSPs to larvae. Here, we determined the age at which adult workers interact with and consume PSPs in an attempt to understand PSP utilization in *Apis mellifera* colonies. We released cohorts of marked, newly emerged workers into hives containing dyed PSPs, undyed PSPs (negative control), dyed fondant (positive control), and dyed modeling clay (consumption control). We recorded the number of marked workers interacting (chewing or extending their proboscises) with the treatments for four weeks following worker release. Additionally, we collected 20 marked workers daily from all colonies to assess the presence of dye in their homogenized guts visually. We constructed generalized linear mixed effect models and used Akaike's Information Criterion (AICc) to perform model selection. Using our top models, we found PSP interaction and consumption gradually increased with worker age until peaking at day 19. These patterns suggest that older workers of pollen handling (10-19 days old) and foraging (18-42 days old) age, rather than nurse bees, are the primary consumers of PSPs. The number and timing of marked bees interacting with and consuming PSPs varied among each colony, indicating individual colony needs may influence PSP utilization. Dye was present in the guts of workers of all ages in hives given dyed fondant, yet worker interactions with dyed fondant followed similar patterns to those of PSPs. Worker age may drive interactions with resources placed inside the hive and fondant, though not PSPs, may be spread through trophallaxis. Our research suggests that PSPs may not provide complete nutritional support for brood production and overall colony health.

### OP-037

#### Validation of Calcein Violet as a marker of *Apis mellifera* semen viability in flow cytometry: Preliminary results

Sophie Egyptien, Flore Brutinel, Benjamin Dewals, Stefan Deleuze

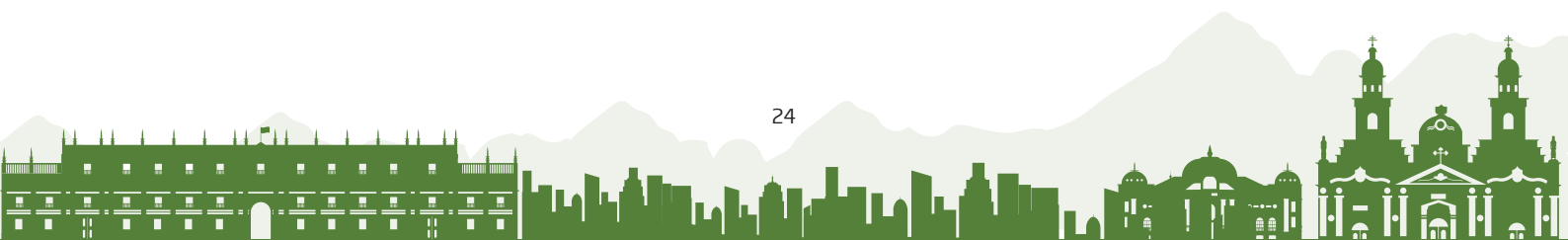
FARAH of Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

Membrane integrity, most commonly assessed by double staining with SYBR®14 (SYBR) and propidium iodide (PI), is considered to reflect viability, which is routinely included in semen evaluation. Many fluorochromes emit in the green and red channels, limiting their possible combinations for multiple parameters analysis. Although previously suggested for that purpose, Calcein violet (CaV), a permeant viability dye that fluoresce after esterification of the non-fluorescent calcein violet acetoxymethyl into fluorescent calcein violet, has recently been validated as a marker of semen viability in honeybee drones (1).

However, compared to flow cytometry, epifluorescence is suboptimal for semen evaluation as it is time consuming and only allows evaluation of a limited number of spermatozoa. This report shows preliminary results on the application of CaV in flow cytometry for viability evaluation of drone semen. Heat-treated samples and unstained samples were used as negative control. Untreated samples showed 84% (73.7-94.3%) of positive cells compared to heat treated samples with 2.5% (1.35-3.7%) of positive cells. Autofluorescence was marginally detected with 0.15% of cells detected as positive on unstained samples.

Although these are preliminary results, this is a decisive first step towards further development with flow cytometry. As CaV fluoresces in violet, it frees the green and red light spectrum channels and broadens the possibilities of combinations to expand the range of parameters simultaneously evaluated in a multiple parameters analysis of semen. This will be particularly beneficial for honeybee drones that have a very small volume of ejaculate and where sample size is an issue. With artificial insemination becoming more and more popular, development of semen quality assessment tools, that have been scientifically validated for the drones, has become crucial for apidology.

(1) Egyptien et al. Validation of calcein violet as a new marker of semen membrane integrity in domestic animals. 2023. Animals. (Under review).



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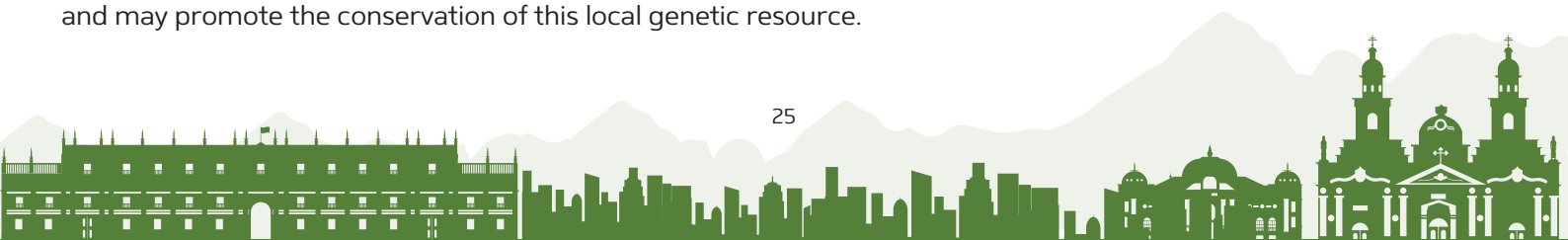
### OP-038

#### Population genetics of a locally domesticated honey bee landrace

Zheguang Lin, Guohong Chen, Ting Ji

Apicultural Research Institute, Yangzhou University, Yangzhou 225009, China

Managed animals translocated by human beings to a non-native distribution area will have to be subject to environmental and anthropogenic challenges, which may affect the population genetics. This is the case for the western honey bee *A. mellifera* that has been introduced to most parts of the world, including Asia. In Northeast China with a long freezing winter, a dark landrace of *A. mellifera*, referred to as the Hunchun bee, was introduced from Europe appr. 100 years ago with indeterminate genetic background and demographic history. Here we sequenced the whole genome of Hunchun bee and analyzed it coupled with the current five *A. mellifera* lineages. Although the results of population genetics revealed a huge divergence between the present lineages and Hunchun bee, C lineage showed the closest relationship, compared to others, with this landrace. Population structure further demonstrated a C lineage dominated ancestry with O lineage admixed. Morphological comparison between Hunchun bee and its potential ancestral subspecies confirmed the population differentiation. These findings indicate a rapid evolutionary rate under local domestication pressure which was uncovered by the high linkage disequilibrium value. Our study fills gaps in the knowledge of the population genetic structure and divergence of the established Hunchun bee landrace and may promote the conservation of this local genetic resource.



### OP-039

#### Africanized honeybee colonies in Nicaragua determined by forewing length and mitochondrial DNA

Christiane Düttmann<sup>1</sup>, Byron Flores Somarriba<sup>1</sup>, Jessica Sheleby-Elias<sup>1</sup>, Gladys Castillo<sup>1</sup>, Daymara Rodríguez Alfonso<sup>2</sup>, Matías Maggi<sup>3</sup>, Jorge Demedio Lorenzo<sup>2</sup>

<sup>1</sup>Escuela de Ciencias Agrarias y Veterinarias, Universidad Nacional Autónoma de Nicaragua, León

<sup>2</sup>Facultad de Medicina Veterinaria, Universidad Agraria de La Habana, Cuba

<sup>3</sup>CONICET Mar del Plata, Universidad Nacional de Mar del Plata, Argentina

Various subspecies of *Apis mellifera* L. were introduced to Central America since the 18th century and genetic introgression increased with the entrance of the Africanized bee in Nicaragua in 1984. Rustic beekeeping activities and numerous feral swarms define the genetic pattern, reflected in phenotypic heterogeneity and remarkable differences in the behaviour of the bee colonies, especially the nest defence. Due to these facts, the question emerge about the degree of Africanization of honeybee colonies in Nicaragua. Morphometric determination based on the single character "mean forewing length" are useful to discriminate easily and rapidly between Africanized and European patterns in honeybee colonies and, subsequently, processing mtDNA analysis using the Dral method, which is highly suitable to discriminate Africanized colonies. In Nicaragua, morphometric and genetic approaches were realized in 26 apiaries in three different geographical zones and related to beehive characteristics and management. Worker bee samples from 146 hives indicated phenotypic heterogeneity in between the colonies (abdominal colour), and the mean forewing length of 8.74 mm (SD 0.16 mm) indicated a high degree of Africanization. Only 28 % of the colonies showed gentle behaviour, but there was no significant difference in defensive behaviour and morphological patterns, however, it was significantly different in the sampled zones. The mitochondrial DNA analysis (bees selected from 61 beehives due to morphological and behavioural characteristics) provided the presence of three mitotypes of the African (A) lineage, one mitotype is still unidentified, whereas 88 bees represented mitotype A1 and 21 bees mitotype A4. Colonies with A4 mitotypes, which are generally characterized by smaller wings, in the present study had longer forewings than bees with A1 mitotype. Changing the protocol, more than one bee was sampled from each hive with the remarkable result that three hives presented bees with both mitotypes A1 and A4. Phylogenetic analysis (sequencing of 10 selected honeybees) confirmed the PCR findings. In addition, no associations were found between mitotypes, forewing length, beehive characteristics and management. A high degree of Africanization in *Apis mellifera* colonies represented by two mitotypes from the A lineage, prevail in Neotropical Nicaragua, with mitotype A4 predominating at higher altitudes.

### OP-040

#### Effects of Different Nutritional Sources on Brood Rearing Capability of Nurse Bees

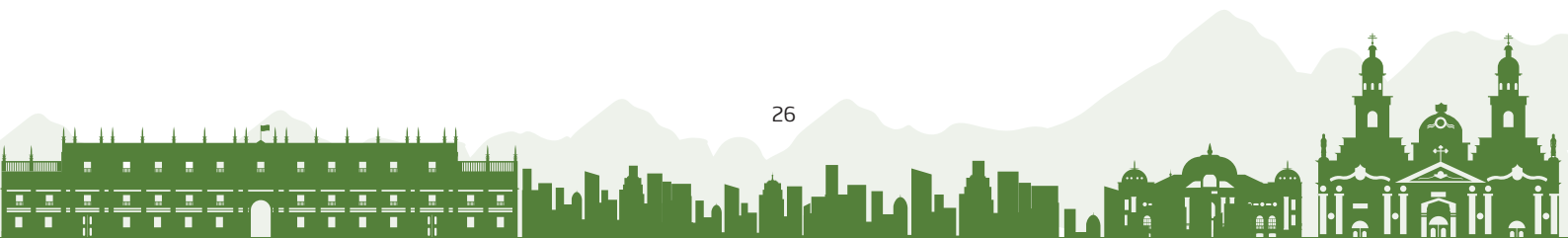
Clara Elisa Castañón, Tiffane Bates, Julia Grassl

Honey Bee Health Research Group, School of Molecular Sciences, The University of Western Australia, Crawley, WA, Australia

The nutritional needs of honey bees during different stages of their lives and labour shifts within the colony are not fully understood, particularly for nurse bees who play a critical role in brood rearing. Existing knowledge on bee nutrition has primarily focused on testing requirements in cages or on adult bees without brood comb. The nutritional needs of nurse bees differ significantly from foragers, as they must develop the hypopharyngeal gland and produce high-quality larval food. The use of substitute feeds is common in the beekeeping industry, but the effectiveness and ideal composition of artificial pollen sources for brood rearing remain unclear.

To address these knowledge gaps, we evaluated the physiological condition of nurse bees fed various supplementary diets in small feeding boxes. Our findings revealed significant differences in the effectiveness and utilization of different feeds, including variations in brood rearing, gland and fat body development, food consumption, and behavioural patterns related to feed management. Molecular analyses such as amino acid, mineral, and vitamin measurement through mass spectrometry are ongoing to determine specific nutrient requirements and toxicity levels in bees.

Our study highlights the critical role of nutritional sources in maintaining the health of nurse bees and promoting brood rearing. The results provide valuable insights into the specific nutritional needs of nurse bees during this important period and could contribute to the development of more effective and targeted nutritional strategies for beekeeping.





### OP-041

#### Molecular identification of stingless bee *Tetragonisca angustula* gut microbiota

Fernanda Giovana Martins de Oliveira, João Arthur Oliveira, Cíntia Zani Fávaro Polonio, Vitor Dib Gazola, Julio Cesar Polonio, Maria Claudia Colla Ruvolo Takasusuki

Department of Biotchnology, Genetcs end Cell Biology, Universidade Estadual de Maringá, Maringá, Brazil

Microbial communities are prominent in the digestive tract of insects, acting as important mediators of the various lifestyles of their hosts, besides providing them with many beneficial services. Although *Tetragonisca angustula* is one of the important stingless bee species (SB) for meliponiculture, and with wide distribution in Brazil, there are few studies about the constitution of the microbiota associated with these bees. Knowing, classifying, and understanding this microbial community will offer new insights into improvements in the health of these SB, on crucial processes of host-microorganism interaction, and on ecological relationships that maintain the balance of populations and their processes within the nest. We aimed to isolate and identify bacteria present in the gut of *T. angustula*. Workers of these SB were collected at the entrance and inside nests. The analyses were performed with the anterior, middle, and posterior regions of their intestines. The strains that grew were identified by sequencing the 16S rDNA gene. Twenty-one bacterial strains were identified, spanning three phyla Firmicutes, Actinobacteria, and Proteobacteria. The predominant phylum was Firmicutes represented by 14 isolates belonging to the Order Bacillales (genera *Paenibacillus*, *Staphylococcus*, *Alkalihalobacillus*, *Bacillus*, *Nialia*, *Terribacillus*, *Lysinibacillus* and *Priestia*); phylum Actinobacteria, one isolate was obtained from the order Micrococcales (*Micrococcus*); and phylum Proteobacteria, six isolates were obtained, one belonging to the order Orbales (*Gilliamella*), two to the order Enterobacterales (*Pantoea* and *Serratia*), and three to the order Pseudomonadales (*Pseudomonas*). We found that there is a difference between the bacteria isolated from the bee's gut present inside and at the entrance of the nests. Bees from the interior of the nest have bacteria from the phyla Firmicutes and Actinobacteria, while bees from the entrance of the nests had in their gut bacteria from all three phyla detected. Eventually, during foraging worker bees may acquire bacteria from the phylum Proteobacteria, since the detected bacterial species from this phylum can be found in the flowers of Angiosperms. These initial results have demonstrated that there is great diversity in the gut microbiota of these SB. They open perspectives for studies on the biology, social behavior, and social immunity of *T. angustula* SB.

### OP-042

#### Alimentary base of *Melipona beecheii* larvae in two urban meliponaries of Cuba

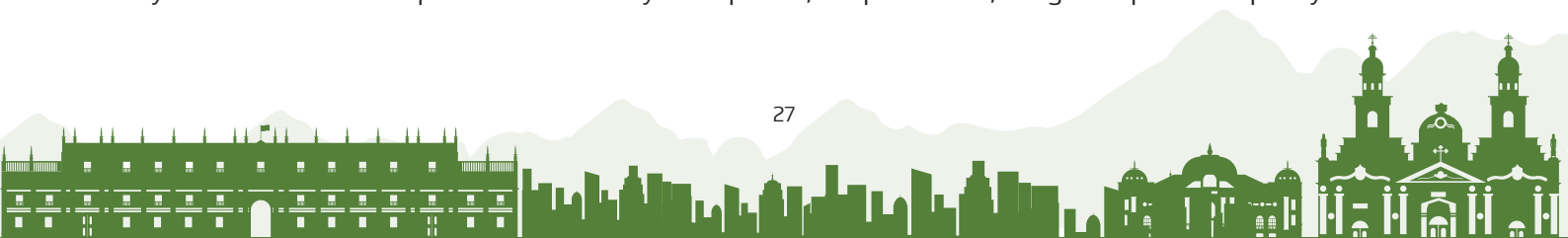
Leydis Fontes-Carballo<sup>1</sup>, Walberto Lóriga Peña<sup>2</sup>, David Rodríguez de la Cruz<sup>3</sup>, José Sánchez Sánchez<sup>3</sup>, Estefanía Sánchez Reyes<sup>3</sup>, Mildrey Soca-Pérez<sup>1</sup>, Jorge Demedio Lorenzo<sup>2</sup>

<sup>1</sup>Estación Experimental de Pastos y Forrajes Indio Hatuey, Universidad de Matanzas, Cuba

<sup>2</sup>Facultad de Medicina Veterinaria, Universidad Agraria de La Habana, Mayabeque, Cuba

<sup>3</sup>Grupo de Palinología y Conservación Vegetal (GPCV), Instituto Hispano Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, España

The nutritional condition during the larval stage is one of the determining factors in the survival of the newly emerged bee. Hence, larval feeding has a fundamental role in the development of the colony. Therefore, this work had as objective to characterize the food base of *Melipona beecheii* Bennett larvae in two meliponaries located in urban areas of Matanzas (MAT) and San Nicolás de Bari (SNB), Mayabeque, 60 km apart. The samplings were carried out in July and December 2019 in 24 randomly selected hives of the Fernando Oliveira/INPA model, 12 in each one. The results showed low diversity ( $H'$ ) and selectivity ( $J'$ ) indices of the hives that showed an oligolectic or specialist behavior for the collection, in accordance with the percentage of pollen predominant in the larval food, which was concentrated in two families, Myrtaceae and Fabaceae. The average protein values obtained in both meliponaries showed higher values in the month of December (19.65 and 20.85 g/100 g, in MAT and SNB, respectively), compared to July (14.64 and 14.62 g/100g). Attributable to higher contributions of the hypopharyngeal glandular secretions of the nurses, due to the fact that in the silage pollen, a fundamental source for the elaboration of this food, there were no differences between the months evaluated. The creation of a "protein bank" close to the meliponary that includes these floral resources of interest to compliment a noticeable part of the protein necessity throughout the year was proposed. It is concluded that the food base of the larvae of *M. beecheii* in the urban environments studied was characterized by the low diversity of the offer and the preference for very few species, despite which, a high adaptative capacity was evident.



### OP-043

#### Will our queens perform?

M Marta Guarna<sup>1</sup>, Leslie Holmes<sup>2</sup>, Shelley Hoover<sup>2</sup>

<sup>1</sup>Department of Agriculture and Agri-Food, Science and Technology, Gov. of Canada, Beaverlodge, AB, Canada, Department of Computer Science, University of Victoria, Victoria, BC, Canada

<sup>2</sup>Department of Biological Sciences, University of Lethbridge, Alberta, Canada

The health and fertility of the honey bee queens are crucial to colony health and productivity. Several factors affects queen health; in this presentation we will review the damaging effect of temperature stress on queen fertility and colony productivity, and report on our recent efforts to develop non-invasive queen fertility tests. In controlled field studies, experiments from us and others have shown that temperature-stressed queens had significantly low productivity and survival. These colonies had poor brood patterns, a fifty percent reduction in adult bee populations, and produced only one-third as much honey compared to control colonies. Further, over half of the colonies headed by temperature-stressed queens perished over the winter. In separate studies, temperature stress was shown to occur during transport, and although queens survived high temperature events, the viability of their stored sperm was significantly reduced. Importantly, queens that suffered damaging effects on fertility from temperature or other stressors show no visible signs of exposure to stress. We will also report on our exploratory experiments to develop non-invasive methods to evaluate queen fertility based on analysis of their fecal microbiome. Increased knowledge of the potential fertility of queens will reduce the proportion of failing queens found in apiaries, and will result in increased confidence when introducing a new batch of queens, particularly if they were transported long distance.

### OP-044

#### Monitoring the circadian activity of solitary bee (*Osmia bicornis*) with RFID technology

Aleksandra Splitt<sup>1</sup>, Mikolaj Boranski<sup>2</sup>, Jacek Jachula<sup>3</sup>

<sup>1</sup>Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland, 2) Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

<sup>2</sup>Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

<sup>3</sup>1) Department of Botany, Mycology and Ecology, Institute of Biological Sciences, Maria Curie-Skłodowska University in Lublin, Poland

2) Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

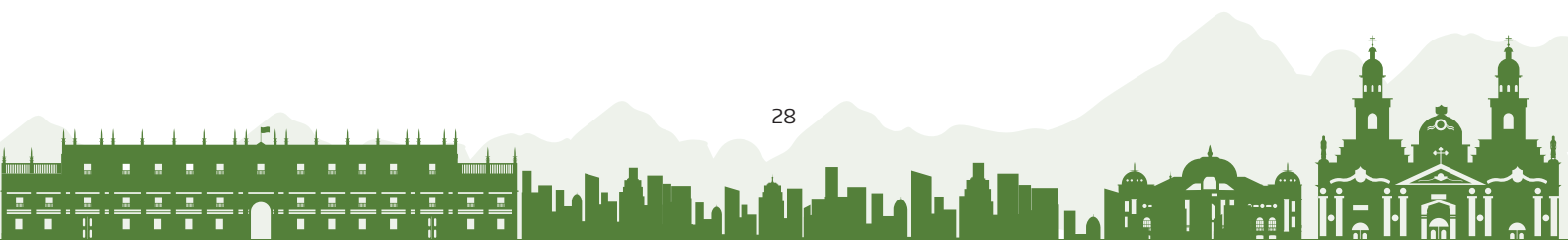
Radio-frequency identification (RFID) is commonly applied technology which uses electromagnetic fields to automatically identify and track tags attached to objects. In the case of bees RFID systems have been mainly used to examine ecology, behaviour and the effects of pesticides in social species e.g. honey bees, bumble bees and stingless bees. To our knowledge so far this method has not been used in solitary bees. We assumed that applying this technology on solitary bees is feasible and can provide a lot of novel, so far unachievable information about their biology.

To trial our assumptions, we have designed and built-up a special nesting construction with RFID system (i.a. antennas network) for solitary cavity nesting bee species – *Osmia bicornis*.

The construction was set up in SE Poland in an early spring. We used special miniaturized passive RFID tags (Hitachi®, Japan) to tag females and males of *O. bicornis*. Every tag was individually coded.

The experiment was successfully conducted, and huge amounts of data has been received from RFID system throughout the 24 hours/day, 7 days/week until the natural death of tagged *O. bicornis*. On a basis of the conducted experiment, it is possible to draw conclusions on the circadian cycle of *O. bicornis* females. Moreover this also shows that it is possible to conduct further future basic research on other species of cavity nesting bees with the usage of RFID technology.

This study was possible through implementation of the research project No. 2019/33/N/NZ8/02864, entitled: “Keeping track of solitary red mason bees flight performance using miniaturized system of radio frequency identification (RFID)” financed by the National Science Centre, Poland.



### OP-045

#### Honey bee nuptials: mating control methods and their efficiency

Janez Prešern<sup>1</sup>, Ajda Moškrič<sup>1</sup>, Andraž Marinc<sup>1</sup>, Katarina Mole<sup>1</sup>, Aleksandar Uzunov<sup>2</sup>, Borče Pavlov<sup>2</sup>, Goran Aleksovski<sup>2</sup>, Marin Kovačić<sup>3</sup>, Zlatko Puškadija<sup>3</sup>, Bjørn Dahle<sup>4</sup>, Jakob Wegener<sup>5</sup>, Ralph Büchler<sup>6</sup>, Sreten Andonov<sup>7</sup>

<sup>1</sup>Agricultural Institute of Slovenia, Ljubljana, Slovenia

<sup>2</sup>CARPEA, Skopje, Macedonia

<sup>3</sup>CALIS, Osijek, Croatia

<sup>4</sup>Norwegian Beekeepers' Association

<sup>5</sup>Institute for Bee Research, Hohen Neuendorf, Germany

<sup>6</sup>Kirchhain, Germany

<sup>7</sup>Swedish University of Agricultural Sciences, Uppsala, Sweden

We have addressed mating control as the key element in selection programs. Honey bee queens mate with drones at the beginning of their adult life in mid-air making it difficult to control their mating partners.

Three different approaches to controlled mating (geographical isolation, isolation by saturation of selected drones a.k.a. biological isolation and temporal isolation a.k.a. moonshine mating) were tested and two groups of parameters were recorded: first group measured the efforts of queens to mate (frequency, duration of nuptial flights); the second group measured success of mating control using paternity assignment. Matching expectations, none of the areas tested in the geographic isolation experiments turned out to be drone-tight, probably due to high colony density in three involved S European countries. However, when the nuptial flight effort parameters were compared between similar locations with or without known presence of other colonies (drone producing colonies, DPCs or other), the values of measured parameters were clearly divided into two groups, the former having shorter successful flights and lower flight frequency (averages 11 - 17 minutes with DPCs vs. 19 - 26 min without DPCs; 1.6 - 2.2 flights with DPCs vs 2.6 - 8.8 flights without DPCs. Furthermore, the biological isolation experiment with 96 DPC returned similar values: average duration of successful mating flight was 14 minutes, owing to the abundance of drones. Temporal isolation, where the experimental queens and drones are released for mating in the late afternoon, was also tested, with the latest successful mating flight as late as 19:55. Paternity assignment was performed using microsatellite multiplex: in biological saturation experiment 93 % of brood was assigned to installed DPCs. In one geographical isolation of Alpine valley 87 % of brood was assigned to the five DPCs present.

The results will be used in the updating the methodology and legislative in beneficiary countries of EEA and Norway Grants Fund for Regional Cooperation project BeeConSel offering local breeders' tools to predict and achieve genetic gain in high colony density situations.

### OP-046

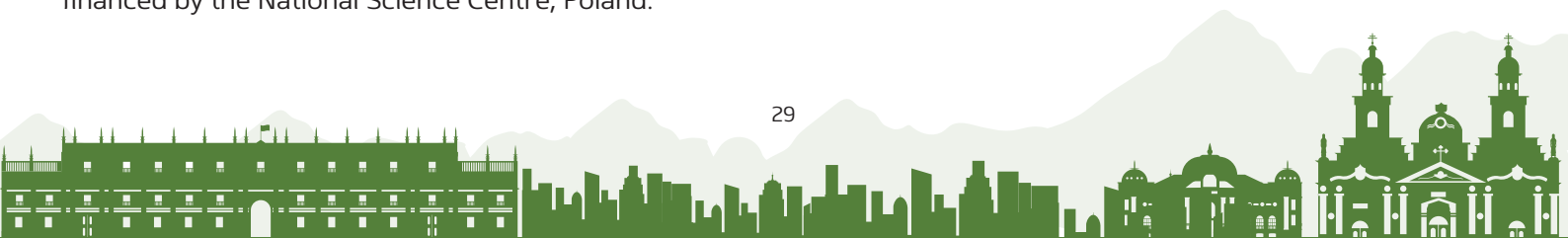
#### Understanding and monitoring social resilience in honeybee colonies

Zeynep Neziha Ulgezen<sup>1</sup>, Coby van Dooremalen<sup>1</sup>, Frank Van Langevelde<sup>2</sup>

<sup>1</sup>Wageningen Plant Research, Wageningen Research, Wageningen, the Netherlands

<sup>2</sup>Wildlife Ecology and Conservation, Wageningen University, Wageningen, the Netherlands

Homeostasis is essential for all organisms, and involves the regulation of balanced internal states for maximizing fitness. In a world of unpredictable perturbations organisms are thought to have developed coping mechanisms to maintain homeostasis. Honeybees are described as superorganisms, where interactions among nestmates and collective behavioral responses regulate the colony. The capacity of superorganisms that allows them to adjust to stress and provides an ecological buffer to negative effects is described as social resilience. Yet, as evidenced with high colony losses, persistence of stress may lead to breakdown in mechanisms, loss in resilience and hinder colony fitness. It is unknown how chronic stress influences social resilience and what mechanisms dictate social resilience in honeybee colonies. In temperate regions, colonies are especially challenged in spring, as they need to show fast growth to prepare for reproduction. Hence, we explored whether bees adjust timing of brood rearing and nest emergence as a coping mechanism in this critical time period, and if there is a loss of social resilience, with consequences on growth and reproduction. For this we monitored chronically stressed (high level of Varroa destructor infestation or nutrition restricted) and presumably non-stressed colonies for over a year. We found that colonies showed a loss of social resilience, leading to diminished growth and reproduction, but were unable to adjust timing of brood rearing and nest emergence. Our study demonstrates a measurable loss of resilience leading to negative effects on colony fitness. We propose that resilience may be a useful measure to monitor colony status. This study was possible through implementation of the research project No. 2019/33/N/NZ8/O2864, entitled: "Keeping track of solitary red mason bees flight performance using miniaturized system of radio frequency identification (RFID)" financed by the National Science Centre, Poland.



### OP-047

## The honey bee pangenome: A key tool for cataloguing and analyzing genetic information

Arián Avalos<sup>1</sup>, Garrett Slater<sup>1</sup>, Andrew Ontano<sup>1</sup>, Justin Vaughn<sup>1</sup>, Brian Scheffler<sup>1</sup>, Anna Childers<sup>1</sup>, Brian Abernathy<sup>2</sup>

<sup>1</sup>United States Department of Agriculture, Agricultural Research Service

<sup>2</sup>University of Georgia

Current understanding of honey bee genome-wide variation is dependent on comparison to a single reference genome. Though traditionally fruitful, this approach also presents clear challenges by constricting our understanding of the magnitude and breath of variation to that available in the reference individual. Ultimately this limits our understanding of honey bee genetic variation. Here we outline the development of a honey bee pangenome, a reference tool able to incorporate genetic variation from multiple individuals. We also provide an accompanying analysis of genetic variation in key research and common-use commercial populations. Our study aims to: (1) introduce a robust framework for the practical use of pangenomes in honey bee research, and (2) to provide tools in the form of graph assemblies and a catalogues of honey bee genetic diversity. Insights from this project will inform future analyses of genome-wide variation and establish powerful tools towards greater understanding of honey bee genetics.

### OP-048

## Brazilian bee traits database

Guaraci D. Cordeiro<sup>1</sup>, Tereza C. Giannini<sup>2</sup>, Patrick M. Consorte<sup>3</sup>, Bruno F. Marques<sup>3</sup>, Nicholas D. Mazzei<sup>3</sup>, Poliana P. Menezes<sup>3</sup>, Antonio J. C. Aguiar<sup>4</sup>, Eduardo A. B. Almeida<sup>5</sup>, Isabel Alves Dos Santos<sup>6</sup>, Tamires O. Andrade<sup>2</sup>, Alistair J. Campbell<sup>7</sup>, Beatriz W. T. Coelho<sup>8</sup>, Rafael R. Ferrari<sup>9</sup>, Elinor M. Lichtenberg<sup>10</sup>, Aline C. Martins<sup>4</sup>, Favízia F. Oliveira<sup>11</sup>, Léo C. Rocha Filho<sup>12</sup>, José E. Santos Junior<sup>13</sup>, Juliana Shimoda<sup>3</sup>, Daniel P. Silva<sup>14</sup>

<sup>1</sup>Universidade Federal de Goiás, Goiania, Brazil, Instituto Tecnológico Vale, Belém, Brazil

<sup>2</sup>Instituto Tecnológico Vale, Belém, Brazil

<sup>3</sup>Universidade Federal de Goiás, Goiania, Brazil

<sup>4</sup>Universidade de Brasília, Brasília, Brazil

<sup>5</sup>Universidade de São Paulo, Ribeirão Preto, Brazil

<sup>6</sup>Universidade de São Paulo, São Paulo, Brazil

<sup>7</sup>Natural England, Kendal, England

<sup>8</sup>Museu Paraense Emilio Goeldi, Belém, Brazil

<sup>9</sup>Chinese Academy of Sciences, Beijing, China

<sup>10</sup>University of North Texas, Denton, United States of America

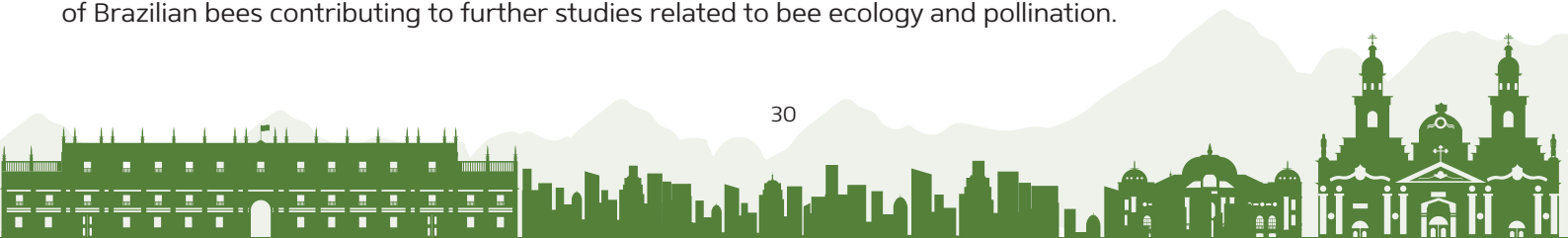
<sup>11</sup>Universidade Federal da Bahia, Salvador, Brazil

<sup>12</sup>Universidade Federal de Uberlândia, Uberlândia, Brazil

<sup>13</sup>Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

<sup>14</sup>Instituto Federal Goiano, Urutaí, Brazil

Brazil has about 10% of the described bee species in the world. However, there is still an enormous Raunkiæran shortfall, with the existing information on species traits being scattered in existing literature and difficult to access. Such shortfall hampers basic scientific research on bee ecology in Brazil. Here we present a Brazilian bee trait database that gathers a large set of available information on the 2043 native species of Brazil presently described in the Moure's Catalog for Neotropical bee species and on recent taxonomy literature. Information on sociality (for 2000 species, 97%), nesting biology (for 1497 species, 73%), and intertegular distance which is a proxy of body size (568 species) were obtained from literature data or via direct measurements. Information on which biomes species occurred was also added. The biome with the highest number of known species was Atlantic Forest with 1375 species (69% of the 2000 species for which we had information on biome of occurrence) followed by Cerrado with 1091 species (55%), Amazon with 1071 species (54%), Caatinga with 572 species (29%), Pampa with 478 species (24%), and Pantanal with 209 species (10%). Most species in Brazil for which we have information on sociality are solitary (938 species;47%) or primitively social (585 species;29%), eusocial representing only 14% of the species (271 species) and cleptoparasitic representing 12% (240 species). Regarding nesting, 714 species (48% of the species for which we had information on nesting biology) build nests above-ground, 710 species (47%) below-ground, and 73 species (5%) are able to nest above or below-ground. Overall, this trait database represents an important advance on the knowledge of Brazilian bees contributing to further studies related to bee ecology and pollination.



### OP-049

#### Genetic characterization of two Algerian honey bees *Apis mellifera intermissa* and *sahariensis* populations by whole genome sequencing

Kamila Canale Tabet<sup>1</sup>, Giovanna Salvatore<sup>2</sup>, Amira Chibani Bahi Amar<sup>3</sup>, Riad Fridi<sup>3</sup>, Nacera Tabet AOUL<sup>3</sup>, Saci Soumia<sup>4</sup>, Emmanuelle Labarthe<sup>1</sup>, Valentino Palombo<sup>2</sup>, Mariasilvia D'ANDREA<sup>2</sup>, Alain Vignal<sup>1</sup>, Pierre Faux<sup>1</sup>

<sup>1</sup>GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France

<sup>2</sup>Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis snc, 86100 Campobasso, Italy

<sup>3</sup>Laboratoire de Génétique Moléculaire et Cellulaire (LGMC), Département de Génétique Moléculaire Appliquée, Université des Sciences et de la Technologie d'Oran Mohamed Boudiaf, USTOMB, BP 1505, El M'naouer, 31000 Oran, Algérie

<sup>4</sup>National Institute of Agronomic Research of Algeria (INRAA), El Harrach, Alger, Algeria

The original geographical distribution of the honey bees *Apis mellifera* in Africa, Europe, and Western Asia, has been extended worldwide through human beekeeping activities. Despite, many studies carried out in order to better understand the genetic diversity of *Apis mellifera* genetic diversity, the genetic composition of some African subspecies is still poorly described. Within the frame of the TSARA initiative, we initiated the study of the Algerian honey bee population, composed of two described subspecies *A. m. intermissa* in the north and *A. m. sahariensis* in the south. Genetic differentiation is expected to match phenotypic specificities and adaptation to heat and drought in *A. m. sahariensis*. Contamination by imported bees is also possible. To shed light on the structure of this population and to integrate these two subspecies in the growing dataset of available haploid drone sequences, we performed whole genome sequencing of 151 haploid drones. Integrated analyses with a dataset of drone sequences from European reference populations did not detect any significant admixture in the Algerian honey bees. Interestingly, most of the genetic variation was not found between the *A. m. intermissa* and *A. m. sahariensis* subspecies and instead, two main genetic clusters were found along an East-West axis. We found that the correlation between genetic and geographic distances was higher in the Western cluster and that close-family relationships were mostly detected in the Eastern cluster, sometimes at long distance. The differences between the two main genetic clusters suggest differential breeding management between Eastern and Western Algeria, with a higher exchange of genetic material over long distances in the East. The lack of detected admixture events suggests that, unlike what is seen in many places worldwide, imports of queens from foreign countries does not seem to have occurred at a large scale in Algeria, a finding of relevance for conservation purposes. The outcomes of our study should be considered in local honey bee biodiversity improvement and conservation initiatives. In the future, we would like to specifically sample and analyse *A. m. sahariensis*, to initiate conservation strategies based on whole genomic data.

### OP-050

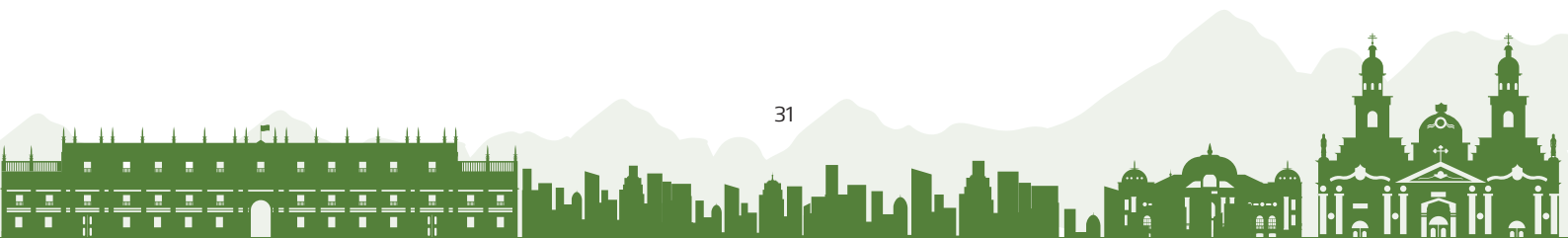
#### Status of Embryo cryopreservation: Studies with the honey bees

Arun Rajamohan<sup>1</sup>, Robert Danka<sup>2</sup>, Kate Ihle<sup>2</sup>, Joseph Rinehart<sup>1</sup>

<sup>1</sup>Insect Genetics and Biochemistry Unit, Edward T. Schafer Agriculture Research Center, USDA-ARS, Fargo, North Dakota, USA

<sup>2</sup>Honey Bee Breeding, Genetics, and Physiology Research Lab, USDA-ARS, Baton Rouge, Louisiana, USA

The urgency to develop new insect germplasm conservation strategies for various bee species and specifically the honey bees (*Apis* sp.) has escalated in recent years especially due to an increase in both the summer and winter colony losses. Contributing factors for colony loss seems to include aspects such as habitat loss, diseases, pesticide stress, management practices, etc., all or some of which seem to culminate in the often-mentioned syndrome of Colony Collapse Disorder (CCD). Our approach up until now has been to strengthen the existing methods for male germplasm cryopreservation. However, the possibility of developing a technique to cryopreserve and store embryonic stages of the bees is enticing considering that such a protocol exists for a dozen other insects species. In this presentation, we will summarize the strategies that we have tested on the honey bee embryos. We will compare the cryopreservation protocols for the honey bees versus the other insect species that have been cryopreserved at the USDA's Agricultural Research Services in Fargo, ND, USA. We will characterize the simplicities and complexities in vitrifying the bee embryos due to their physical and physiological parameters that differentiates these embryos from other insect species. Till date, studies on characterization of the bee embryos show that their amenability to previously published vitrification techniques for dipterans are thwarted by the embryos' solvent uptake characteristics, their membrane fragility, rate of consumption of internal lipid resources and their dynamic chemical toxicity tolerance. Despite that this study adopts the stage-selection technique that was developed for the dipterans. Using this method,  $21.1 \pm 15.4\%$  of the honey bee embryos that were vitrified could be revived. The study estimated that the proportion of embryos that could be carried into the cryoprotectant loading phase of the treatment was merely  $34.5 \pm 9.1\%$ . This indicates the most deleterious effects were due to the permeabilization process. This places a constraint on the possibility of an unified template for insect embryo cryopreservation. In this presentation, the authors will elaborate on the reasoning for such a conclusion and will discuss as well as welcome discussion on how this project could be furthered.





### OP-051

#### Apparent digestibility of lipids and fatty acids by *Apis mellifera* (L.): a comparative study of different bee pollen types collected in Argentina

Matías Francisco Ledesma González<sup>1</sup>, Gustavo López<sup>2</sup>, Gisela Grandinetti<sup>3</sup>, Elián Tourn<sup>4</sup>, Leticia Andrea Fernández<sup>5</sup>, Francisco José Reynaldi<sup>6</sup>

<sup>1</sup>Laboratorio de Estudios Apícolas (LabEA-CIC), Departamento de Agronomía, Universidad Nacional del Sur (UNS). Bahía Blanca, Prov. Buenos Aires, Argentina - Estudiante de doctorado, Becas Doctorales Temas Estratégicos CONICET

<sup>2</sup>Cátedra de Bionalítica II. Departamento de Biología, Bioquímica y Farmacia. UNS.

<sup>3</sup>Laboratorio de Estudios Apícolas (LabEA-CIC).

<sup>4</sup>Laboratorio de Estudios Apícolas (LabEA-CIC) - Cooperativa de Trabajo Apícola Pampero Ltda. (CAP)

<sup>5</sup>Laboratorio de Estudios Apícolas (LabEA-CIC) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

<sup>6</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Centro de Microbiología Básica y Aplicada (CEMIBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (UNLP). La Plata, Prov. Buenos Aires, Argentina

Pollen is the primary natural source of proteins and lipids for *Apis mellifera* and it plays an essential role in bee nutrition. In this study we measured in vitro apparent digestibility of eight bee pollen (Brassicaceae, Eucalyptus sp., Rhamnaceae, Neltuma sp., Carduus sp., Schinus sp., Pinus sp. [hand collected] and Prunus sp.) with focus in lipid content and fatty acids profiles. Bee pollen were collected in four locations in the southwest of Buenos Aires province. Newly hatched bees were randomly placed in cages (with 50 bees per cage) at 34°C and 60% relative humidity. Pollen was provided fresh with the addition of TiO<sub>2</sub> as inert marker (0.1% w/w based on dry mass basis). Faeces were collected by dissecting the insect's rectum after 14 days and were used to obtain bee pollen apparent digestibility and nutrient apparent digestibility. Lipid content was quantified by Goldfish method while fatty acid profile by gas chromatography. Statistically significant differences were observed in bee pollen apparent digestibility. Carduus sp. (71.72% ±5.3) had the highest bee pollen apparent digestibility values, and Brassicaceae (60.92% ±10.05) and Pinus sp. (5.39% ±41.3) had the lowest ones. Faeces had a higher content of total lipids (30.27% ±17.8) in relation to bee pollen (3.38% ±1.9). Lipid apparent digestibility was 95.30% ±5.5, highest score for Brassicaceae (98.24%) and lowest for Pinus sp. (81.94%). The main fatty acids in faeces were oleic (C18:1), linoleic (C18:2), palmitic (C16:0) and linolenic (C18:3) acid similar to those in bee pollen. Faeces had C18:1 as the main fatty acid and had lower concentration of C16:0, C18:3 and C18:2 than their respective pollens. Main fatty acid apparent digestibility was 84,79% for C18:1, 88,70% for C18:2, 95,38% for C16:0 and 89,32% for C18:3. We concluded that lipids and their fatty acids profiles undergo changes as they pass through digestive tract. Lipids had apparent digestibility near 100% showing that they are highly digestible nutrient for honeybee. These results could be used as a starting point for improving beekeeping practices in order to take advantages of different types of pollens.

### OP-052

#### "Antennal and behavioural responses of honeybees, *Apis mellifera*, to avocado flower volatiles"

Mario Alberto Marsiglia Lans<sup>1</sup>, Jan Bergmann<sup>1</sup>, Sylvia Anton<sup>3</sup>, Jaime Martinez Harms<sup>2</sup>

<sup>1</sup>Instituto de Química, Pontificia Universidad Católica de Valparaíso, Avda. Universidad 330, Valparaíso, Chile.

<sup>2</sup>Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Chorrillos 86, La Cruz, Valparaíso, Chile

<sup>3</sup>Institut de Génétique, Environnement et Protection des Plantes (IGEPP), Institut National de la Recherche Agronomique (INRA), 2, rue André Le Nôtre F-49045 Angers, Francia.

Flower-emitted volatile organic compounds (VOCs) play an important role in the attraction of pollinating insects. To improve pollinating services, it is important to understand which VOCs are detected by potential pollinating species and if individual compounds or specific mixtures improve insect attraction. We have previously identified VOCs emitted by avocado flowers, including among others (E)- and (Z)-4,8-dimethyl-1,3,7-nonatriene, eucalyptol, sabinene, ocimene, pinene, benzyl cyanide, mircene, limonene and linalool. Using electroantennogram recordings, we investigated whether the identified individual compounds are detected by the antennae of honey bees, "*Apis mellifera*", and tested various mixtures of compounds in behavioural choice experiments. Our study provides basic knowledge for the development of synthetic mixtures of VOCs attracting different species of pollinators based on compounds naturally emitted by avocado flowers. This new strategy will help to improve pollinator resource management for various crops and improve production, without introducing new chemical compounds to the environment, which contributes to the knowledge of green chemistry.



### OP-053

#### Nutritional enrichment of pollen for honey bee diet through the internal and external processes by honey bees

Sampat Ghosh<sup>1</sup>, Chuleui Jung<sup>2</sup>

<sup>1</sup>Agriculture Science and Technology Research Institute, Andong National University, Andong, South Korea

<sup>2</sup>Agriculture Science and Technology Research Institute, Andong National University, Andong, South Korea, Department of Plant Medical, Andong National University, Andong, South Korea

Honey bees rely on nectar and pollen for their nutrition, with pollen serving as the primary source of protein, lipid, and micronutrients. While the nutritional reward is a crucial factor in their foraging decisions, honey bees do not consume the fresh collected pollen; instead, they process it into bee bread. We investigated the whole process from the foraging behavior of honey bee, nutritional changes from fresh pollen to bee-pollen, pollen patty, bee bread, and even royal jelly. Our findings suggested honey bee foragers adjust their pollen collection based on the colony's needs and preferences, particularly with regard to amino acids and protein content. The protein (and amino acid), and fat (and fatty acids) contents of pollen patties were found to be much lower than these of bee pollen, while the opposite was true for carbohydrate content, presumably due to the addition of sucrose in pollen patties. The total and individual amino acid contents of bee bread, especially proline, increased significantly. Based on our investigation of the bacterial population of bee pollen and bee bread as well as the existing knowledge about the honey bee gut symbionts, we found that there is scope for nutrition provisioning by the bacteria. They may play a crucial role in nutrient manipulation, which could potentially impact the bee's nutrition. Additionally, our investigation revealed that the nutritional variation in food could affect the quality of royal jelly produced by nurse bees.

### OP-054

#### Behavioural studies into the brain-gut axis of honey bees: How gut microbes can affect complex social behaviour of hives

Sophie M Killam<sup>1</sup>, Anna M Chernyshova<sup>1</sup>, Brendan A Daisley<sup>2</sup>, Andrew P Pitek<sup>1</sup>, Julia F Lacika<sup>1</sup>, Shenella A George<sup>1</sup>, Jeremy P Burton<sup>3</sup>, Emma Allen-Vercoe<sup>2</sup>, Graham J Thompson<sup>1</sup>

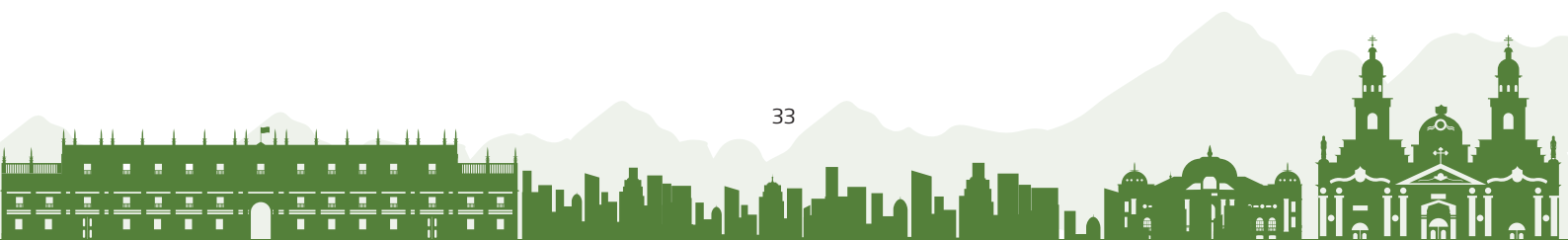
<sup>1</sup>Department of Biology, Western University, Canada

<sup>2</sup>Department of Molecular and Cellular Biology, University of Guelph, Canada

<sup>3</sup>Department of Microbiology and Immunology, Western University, Canada

The evolutionary association between multicellular hosts and unicellular gut microbes represents a deeply functional symbiosis that is mediated in part by a brain-gut axis. This pathway links the metabolic function of microbes within the gastrointestinal tract (i.e., the gut) to the central nervous system (i.e., the brain) and thus to the performance and behaviour of the organism. Studies on the brain-gut axis have been primarily applied to humans and other vertebrate animals but research has begun to extend the concept to invertebrate hosts, including insects. Social insects like honey bees make interesting case studies because of the potential for gut microbial interventions to affect individual and colony-level behaviour. We have begun testing this idea through a series of field studies that use probiotic and antibiotic treatments to manipulate the honey bee brain-gut axis, followed by monitoring of social behaviour of hives.

First, we have developed two modes of delivery that serve to dose colonies with regulated concentrations of (mostly) lactic acid-producing bacteria. We show that oral and topical applications can duly alter gut and, in some cases, immune and brain markers that suggests experimental access to the brain-gut axis of honey bee workers. Second, our use of defense (stinging), foraging (pollen and nectar) and hygiene (uncap and remove freeze-killed brood) assays provide the best test yet of how responsive is the social labour of workers to colony-wide manipulations of their gut microbiota. Our results so far indicate that defence, foraging and hygienic behaviour – all performed by individual workers to the benefit of nestmates – do respond to pro- and anti-biotic treatments, and do so in potentially manageable ways. Our work therefore exploits natural variation in bee-microbe relations to enhance the worker-led performance of hives.



### OP-055

## Use of four modified diluents as alternative diluents for the refrigeration of semen from africanized honeybee (*Apis mellifera*) drones

Katia Peres Gramacho<sup>1</sup>, Lucas Da Silva Morais<sup>1</sup>, Andréia Maria da Silva<sup>1</sup>, Edgar Rodrigues De Araujo Neto<sup>1</sup>, Ana F. S. Cunha<sup>2</sup>, Luana G. P. Bezerra<sup>1</sup>, Nailton Chagas<sup>1</sup>, Leandro Alves Da Silva<sup>1</sup>, Tuanny D. A. Gomes<sup>1</sup>, Francisco Assis Junior<sup>1</sup>, Herica Tertulino Domingues<sup>1</sup>, Jonas Vasconcelos<sup>1</sup>, João BatistaFreire Souza-Junior<sup>1</sup>, Alexandre Rodrigues Silva<sup>1</sup>, Débora Façanha<sup>3</sup>

<sup>1</sup>Lucas S. Morais

<sup>2</sup>Andréia M. Silva

<sup>3</sup>Edgar R. Araujo Neto

<sup>4</sup>Ana F. S. Cunha

<sup>5</sup>Luana G. P. Bezerra

<sup>6</sup>Nailton O. S. Chagas

<sup>7</sup>Leandro A. Silva

<sup>8</sup>Tuanny D. A. Gomes

<sup>9</sup>Francisco Assis Junior

<sup>10</sup>Hérica G. T. Domingos

<sup>11</sup>Jonas E. Vasconcelos Filho

<sup>12</sup>João B. F. Souza Júnior

<sup>13</sup>Alexandre R. Silva

<sup>14</sup>Débora A. E. Façanha

<sup>15</sup>Katia P. Gramacho

Diluents play a crucial role in semen processing and are indispensable for ensuring the viability of male gametes during transportation and storage. The main objective of these media is to protect sperm from the negative effects of temperature reduction and the injuries that may occur during these processes. Thus, the aim of this study was to evaluate the effects of four diluents on the spermatic aspects of drone semen subjected to refrigeration for 96 hours. Three modified diluents, Tris, Tris + egg yolk, Collins, and Ringer, using a dilution ratio of 12:1 (diluent: semen) were tested. For refrigeration, samples were incubated in a BOD at  $16 \pm 1^\circ\text{C}$  and evaluated at 0, 24, 48, 72, and 96 hours after collection. Sperm motility was subjectively evaluated, whereas sperm viability was assessed using Hoechst 33342 and propidium iodide fluorescent probes. The results are expressed as the mean  $\pm$  standard error. The influence of different diluents and incubation times was evaluated by two-way ANOVA, followed by Tukey's test ( $p < 0.05$ ). Fresh semen presented an average value of  $95.7 \pm 1.7\%$  motility and  $71.8 \pm 4.6\%$  sperm viability. There was a significant interaction between the sperm parameters over time among the studied diluents. Ringer diluent showed total motility loss from 24 hours, while the others showed motility up to 96 hours,  $11.4 \pm 2.8\%$  and  $25 \pm 4.2\%$  for Tris and Tris + egg yolk and  $2.6 \pm 0.9\%$  for Collins. Viability showed significant differences from 72 hours, being  $68.1 \pm 5.1\%$  and  $41.7 \pm 9.3\%$  for Tris and Tris + egg yolk and  $56 \pm 5.2\%$  and  $52.8 \pm 3\%$  for Collins and Ringer. Therefore, these are important findings to be considered when using germplasm from endangered populations in the need to create biobanks and new technologies for species conservation.





### OP-056

#### **Stress drives premature self-removal behavior that leads to death in young honey bee (*Apis mellifera*) workers**

Jordan Twombly Ellis, Juliana Rangel

Department of Entomology Texas A&M University

The Western honey bee, *Apis mellifera*, is an economically important pollinator and a tractable species for studying the behavioral intricacies of eusociality. Honey bees are currently challenged by multiple biotic and environmental stressors, many of which act concomitantly to affect colony health and productivity. For instance, stress can lead workers to become precocious foragers and leave the colony prematurely. Precocious foragers have decreased flight time and lower foraging efficiency, which can ultimately lead to colony collapse. In this study, we tested the hypothesis that stress during pupal development can cause young workers to exit their colony prematurely before they can fly. This premature self-removal behavior results in death outside the hive soon after. To determine how various stressors may lead to bees performing this behavior, we subjected workers during the last pupal stage to either cold (26°C for 24 h), hot (39°C for 24 h), or Varroa mite stress, and compared the rate of premature self-removal between stressed bees and respective control counterparts. Upon emergence, we individually tagged focal bees in all treatment groups and introduced them to an observation hive. We then followed tagged bees over time and monitored survivorship and performance of premature self-removal behavior. We found that significantly more bees in all three treatment groups self-removed prematurely compared to their control counterparts. Bees in all treatment groups also had significantly smaller hypopharyngeal glands than control bees, suggesting that premature self-removal is a stress-driven behavior, and potentially a form of accelerated age polyethism that leads to premature death.

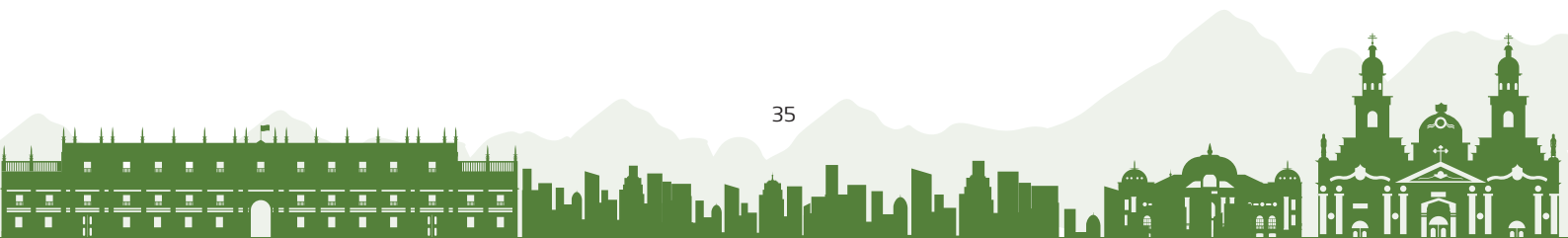
### OP-057

#### **"Rational management of the world's smallest bee (*Leurotrigona Muelleri*) and its impact on native forests in protected natural areas"**

Juan Richard Barja Ñaupari

Company: Bioapinativa, Ucayali, Peru

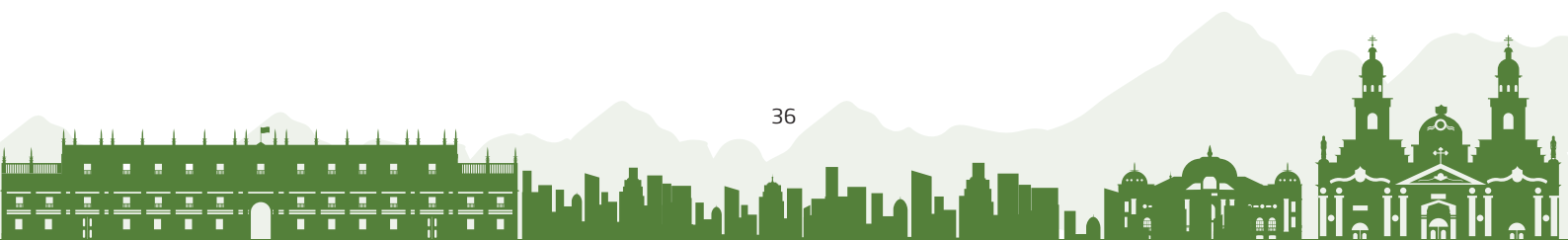
The natural protected areas of Purús are the largest and most preserved in all of Peru, with a mega diversity of species among which the native stingless bees stand out, for which many management and research studies are still lacking. The present study was developed on the species *Leurotrigona Muelleri*, (Friese, 1900). The smallest native bees in the world, commonly known as mosquito bees or eye lickers, these stingless native bees belonging to the group of minor bees of the Amazon tropics, have a great impact on native forests because they are specialists in their pollination, initially described by Blumenau, Santa Catarina, Brazil (Friese, 1900), the nests they develop are small cavities (Pedro & Camargo, 2009). Registered in the natural protected areas of Purús, Ucayali, Peru. The aspects developed in the study are; techniques for rational management, biology and identification of native flora, since due to the delicacy of their hatchling eggs and the size of 1.7 to 3 mm, special care is needed in the adaptation and management in rational nests. The objective is the sustainable use of its products, ecosystem services (pollination) that are of vital importance in the conservation of the native flora and fauna of protected natural areas and insertion in children's environmental education in native communities and deeper studies.



Klaus Hartfelder

Ribeirão Preto Medical School – University of São Paulo

Worker sterility is a fundamental issue in the evolution of complex social systems of insects. While kin selection theory removed the apparent conflict for Darwinian theory to explain the occurrence of a sterile worker caste, the mechanistic underpinnings are still largely unknown. In the honey bee, *Apis mellifera*, massive cell death destroys the majority of the ovarioles as the worker larvae prepare for metamorphosis, and in the adult workers, the activation of the remaining ovarioles is kept in check by the queen's pheromones. Here we present evidence for an epigenetic mechanism that acts at both stages in the life cycle of a worker. We show that the long noncoding RNA *Incov1* physically interacts with the Tudor staphylococcus nuclease (Tudor-SN) protein to form a regulatory module that activates the effector protein caspase, driving cell death in the ovaries of the worker larvae. In adult workers, the *Incov1*/Tudor-SN module then flexibly responds to social cues (queen pheromone and diet) that knowingly reduce their reproductive capacity. Through searches across the genomes of social Hymenoptera we found that *Incov1* is surprisingly conserved in the corbiculate bees, and to a considerable extent also in the other tribes of the Apidae, but not in wasps or ants. Hence, we propose that, by promoting worker sterility, the *Incov1*/Tudor-SN module has likely played an important role in the social evolution of the bees.



### OP-059

#### The honey bees immune memory

**Rodrigo Aarón Burciaga<sup>1</sup>, Gloria Ruiz Guzman<sup>1</sup>, Humberto Lanz Mendoza<sup>2</sup>, Indrikis Krams<sup>3</sup>, Jorge Contreras Garduño<sup>1</sup>**

<sup>1</sup>ENES, Unidad Morelia, UNAM. Antigua Carretera a Patzcuaro, No.8701. Col. Ex-Hacienda San José de la Huerta

Código, 58190, Morelia, Michoacán, México

<sup>2</sup>Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico

<sup>3</sup>Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia

Invertebrates' immune priming or innate immune memory is an analogous response to the vertebrates' adaptive memory. We investigated if honey bees have immune memory. We compared survival and immune response between bees that were: 1) manipulated (Naïve), 2) challenged twice with the same pathogen *Escherichia coli* (Memory), 3) challenged twice with different pathogens (*Staphylococcus aureus* versus *E. coli*, *Micrococcus lysodeikticus* versus *E. coli*), or 4) with PBS (the diluent of bacteria) versus *E. coli* (heterologous challenge; Control). Results indicate better survival in the Memory than the Control group, and the Memory group showed a similar survival than Naïve insects. The Memory group had higher lytic activity but lower prophenoloxidase, phenoloxidase activity, and hemocyte count than the Control and Naïve groups. No differences were found in relative expression of defensin-1. This first demonstration of immune memory opens the questions about its molecular mechanisms and whether, immune memory could be used against natural parasites that affect honey bees, hence, if they could be "vaccinated" against some natural parasites.

### OP-060

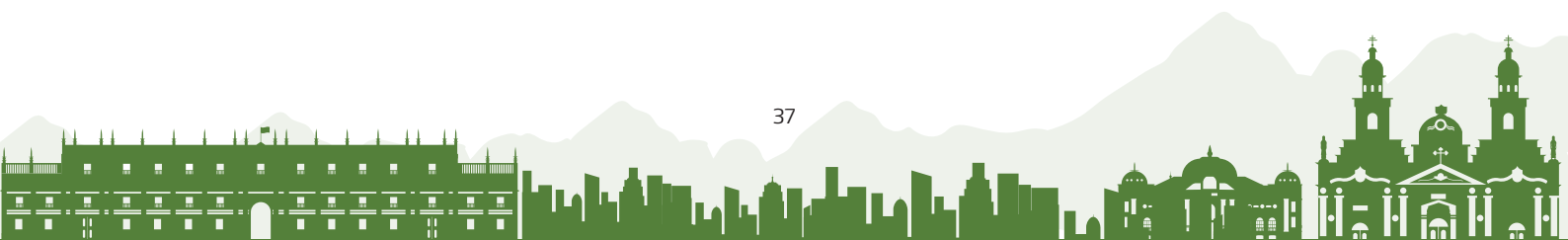
#### Chronic exposure to sulfoxaflor and thiamethoxam reduces sperm viability in Western honey bee drones

**Orlando Yanez, Alexander Cotting, Lars Straub, Verena Strobl, Peter Neumann**

Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Drones are indispensable for honey bee colony functionality as their sperm is essential for the production of female offspring by queens. Agrochemicals acting alone or in combination can impact drone survival and sperm viability thereby having a negative impact on colony health. However, the role of chemical cocktails and of novel substances is often poorly understood. Here, we examined in the laboratory chronically-exposed drones caged with workers to investigate the effects of field-realistic concentrations of the sulfoximine-based insecticide sulfoxaflor (SFX) and the neonicotinoid thiamethoxam (THX), alone or in combination, on drone survival and sperm traits.

Sperm assessments showed that SFX and THX alone and in combination significantly impaired sperm viability compared to the controls. On the other hand, there was no significant effect on drone mortality from any of the treatments. The data support that adverse effects of pesticides on sperm may contribute to colony losses due to failing queens. The results further suggest that a focus on mortality alone bears the danger of false negative results. We therefore suggest that sub-lethal effects on male bee fertility should be included in environmental risk assessments for such agrochemicals.



### OP-061

## Identification of pesticide residues found in dead bees between 2020 and 2022, in the State of São Paulo, Brazil

Renata Sordi Taveira<sup>1</sup>, Maria Carolina Guido<sup>1</sup>, Simael Rosim<sup>1</sup>, Ane Beatriz Camargo Veronez<sup>1</sup>, Eliane Vieira<sup>2</sup>

<sup>1</sup>Coordination of Agricultural Defense

<sup>2</sup>Biological Institute

Bees are insects playing an important role in the preservation of ecosystems and food production, being the main pollinators of native vegetation areas and crops. The inappropriate use of pesticides can cause severe stress to this population, resulting in acute or chronic mortality among *Apis mellifera* bees and Brazilian native bees, in addition to subsequent economic losses. This work aims to report the main pesticide residues found in dead bees in apiaries in São Paulo, Brazil. In the State of São Paulo, Resolution No. 41/2019 of the Secretariat of Agriculture and Supply (SAA), establishes the mandatory notification of acute bee mortality and the care of these cases by the Coordination of Agricultural Defense (CDA). Between 2020 and 2022, 55 cases of mortality involving *Apis mellifera* and seven cases involving native bees were reported, accounting for a total of 2,729 hives. Inspection of notified cases involves the visit of CDA Veterinarians and Agronomists to the location. If the suspicion of mortality due to intoxication is justified, the collection of dead and dying bees is carried out for analysis at the Agrochemical Ecology Laboratory of the Biological Institute at the SAA. Of the total of 62 cases evaluated in these three years, residues were not detected in ten samples, and in three samples it was not possible to collect biological material for analysis. Of the 49 samples with residue detection, the most common active ingredients were fipronil (63%), bifenthrin (22%), trifloxystrobin (20%), and tebuconazole (18%), associated or not with other residues, in values above and below the LD50 described in the literature. The insecticide fipronil was the active ingredient found most frequently (63%), but other classes of use were also detected, such as acaricides, fungicides, and herbicides, suggesting the potential damage of pesticides in impairing the behavior and development of colonies. Notifications of bee mortality by beekeepers allow the Public Service to assess the main critical locations where bee intoxication occurs, as well as directly inspecting and subsidizing public policies for the protection and preservation of bees in pollination and honey production.

### OP-062

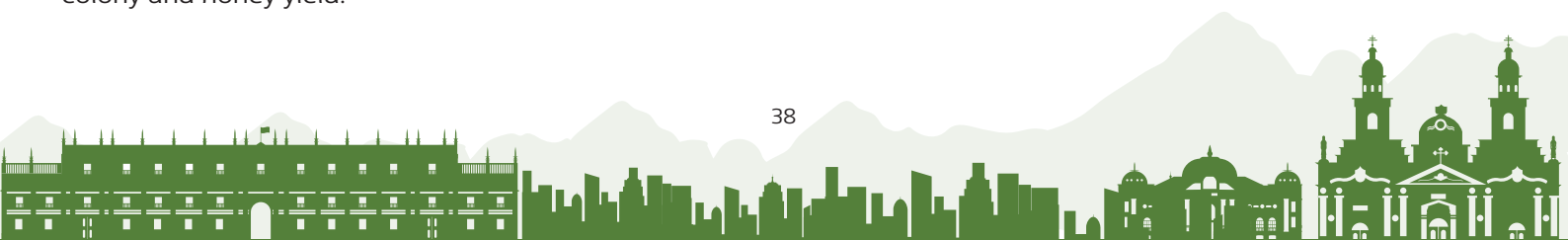
## The application of the comet assay to assess the genotoxicity of insecticides in honey bees

Samina Qamer<sup>1</sup>, Muhammad Naveed Akhtar<sup>1</sup>, Muhammad Sajid<sup>1</sup>, Zulfiqar Haider<sup>1</sup>, Mazhar Farid<sup>1</sup>, Usman Jamal<sup>1</sup>, Nawishtay Rehman<sup>2</sup>

<sup>1</sup>Department of Zoology, Government College University Faisalabad, Pakistan

<sup>2</sup>RIPHAH International University Faisalabad Campus, Faisalabad, Pakistan

The objective of the research work was to study the effects of insecticides used in fields, on adult *A. mellifera* by determining the DNA damage to the eukaryotic cell's nucleus. Forager worker bees of *A. mellifera* species samples (bees exposed to insecticide/Pesticide in field) were collected from the entrance of the bee hives in district Nankana Sahib, Sialkot, Hafiz Abad, Mandi Bhauddin, Chiniot, Gujranwala, Narowal, Sragodha, Khishab and Kasure. Hemolymph of these honeybee's samples was ejected by inserting capillary tube between 4th and 5th abdominal segments. Layering of slides was performed using 1% NMP agarose. The single cell gel electrophoresis or comet assay was used to measure damaged DNA in every hemocytes through Epifluorescent microscope. Three parameters i-e Tail Length, Tail DNA and Olive Tail Movement were used to assess the DNA damage caused by insecticides/Pesticides. The results indicated that overall maximum DNA damage was found in honeybees collected from district Khushab. It showed maximum length of comet head (29.05µm), length of tail (26.01µm), length of comet (55.15µm) and tail movement (17.99µm). The minimum genotoxicity was recorded in bees from Nankana sahib area as it had maximum head DNA length (72.49µm). Olive tail movement from Narowal (7.32µm). It can be concluded that indiscriminate use of insecticides or pesticides in agricultural fields or near honey bees apiaries caused not only the DNA damage to them but also affects the bee's orientation and other physiological behavior. This in turn impact the strength of the bee colony and honey yield.



### OP-063

## Sentinel apiaries, epidemiological surveillance and training programs as strategies for early detection of the Small Hive Beetle in Africanized honey bees in Costa Rica

Rafael Calderon<sup>1</sup>, Luis Sánchez<sup>2</sup>

<sup>1</sup>Programa Integrado de Patología Apícola, Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, Heredia, Costa Rica.

<sup>2</sup>Programa Integrado de Ecología y Polinización, Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, Heredia, Costa Rica.

The small hive beetle (SHB), (*Aethina tumida*) Murray, is a parasite and scavenger of honey bee (*Apis mellifera*) colonies endemic to sub-Saharan Africa. It has been found in Africanized honey bee (AHB) colonies in several countries of North and South America. In Central America, it was discovered in El Salvador in 2013 and in Nicaragua in March 2014. In Nicaragua, the SHB was confirmed in AHB colonies located in San Juan del Sur, Department of Rivas, about eight kilometers north of the border with Costa Rica, increases the risk of invasion of this pest into bee hives in Costa Rica. Following the confirmation of SHB presence in Nicaragua a “sentinel apiary” with four AHB colonies was established in Santa Cecilia, La Cruz, province of Guanacaste, Costa Rica, close to the border. Colonies were monitored visually by examining all individual frames, hive covers, and bottom boards. SHBs were detected and confirmed in the sentinel apiary in August 2015. Only adult beetles were detected in the AHB colonies. After the confirmation in the north of Costa Rica, an epidemiological surveillance of the main beekeeping areas was conducted during the last years (2015 - 2022) to monitor the distribution. In addition, training programs were directed to technicians and beekeepers focused on SHB recognition and identification, and methods for colony inspection. These programs included workshops, fieldwork and training materials flyers and brochures. The SHB has been found in different commercial apiaries in collaboration with trained beekeepers, especially in the province of Guanacaste. In conclusion, implementing strategies for the early detection of SHB as it spreads to new countries or areas requires, as illustrated by the case in Costa Rica, implementing sentinel apiaries, and the development of epidemiological surveillance, and training activities for technicians and beekeepers to aid in beetle recognition.

### OP-064

## Engaging farmers and pesticide applicators in pollinator stewardship

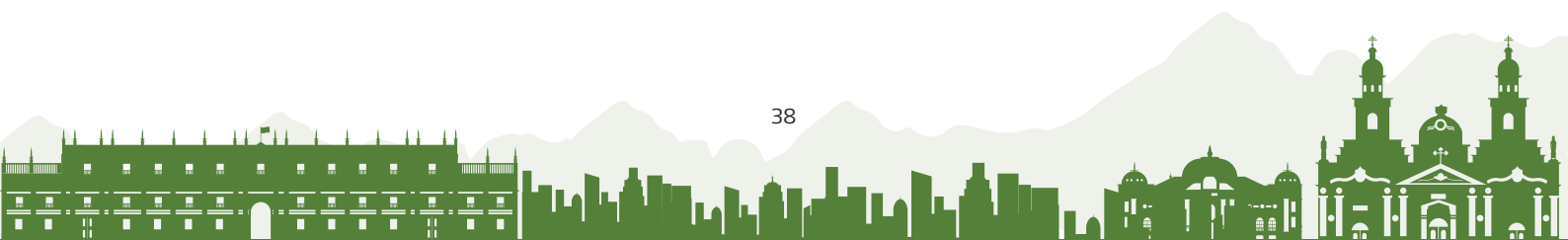
Ana Heck

Michigan State University

Honey bees need all the help they can get. Farmers, growers, land managers, and other pesticide applicators are blamed for harming honey bees, but these groups are often full of individuals who are eager to help. Ana Heck, Apiculture Extension Educator at Michigan State University, will present on programs at the state and national level that provide messaging, outreach, and resources on pollinator protection to pesticide applicators.

Heck will share specific examples of pollinator stewardship programs in Michigan, USA. Michigan is home to several specialty crops that depend on honey bees for pollination but also require intense pest management. The pollinator stewardship programs aim to support pollinator health by engaging farmers, growers, and land managers to plant for pollinators and use pesticides judiciously.

Heck leads the national Managed Pollinator Protection Plans Working Group ([www.canr.msu.edu/resources/national-mp3-working-group](http://www.canr.msu.edu/resources/national-mp3-working-group)), which is funded by the North Central IPM Center. The group includes about 40 extension educators and specialists, apiary inspectors, and professionals from state and national agencies who work together to share resources and develop educational materials to promote pollinator stewardship. The working group developed presentations and online courses to train pesticide applicators in pollinator protection. The training materials topics include pollinator appreciation, pesticide toxicity and risk to bees, pesticide labels, and planting for bees. The working group aims to provide practical, science-based recommendations to pesticide applicators that they can be part of the bee health solution.



### OP-065

## A new frontier for visualising the impact of stressors in bees: proteins pictured by mass spectrometry

Philippe Bulet<sup>1</sup>, Camille Houdelet<sup>2</sup>, Karim Arafah<sup>3</sup>, Michel Bocquet<sup>4</sup>, Cédric Alaux<sup>5</sup>, Anne Bonjour Dalmon<sup>5</sup>, Léna Barascou<sup>5</sup>, Yves Le Conte<sup>5</sup>, Deborah Sene<sup>5</sup>

<sup>1</sup>University Grenoble Alpes, Inserm U 1209, CNRS UMR 5309, Institute for Advanced Biosciences, 38000 Grenoble, France

<sup>2</sup>UMR1419 Nutrition, Métabolisme, Aquaculture (NuMéA), Aquapôle INRAE, 64310 Saint Pée sur Nivelle, France

<sup>3</sup>Plateforme BioPark d'Archamps, Archamps, France

<sup>4</sup>Apimedia, Pringy, Annecy, France

<sup>5</sup>NRAE, Abeilles et Environnement, Avignon, France

Among pollinating insects, bees play a critical role in boosting reproduction of wild and commercial plants, thus contributing to the preservation of plant biodiversity and sustainability of food webs. In the last few decades, domesticated and wild bees have been subjected to biotic and abiotic threats causing various health disorders. This presentation will focus on nosemosis, an infection caused by the single-cell microsporidian parasite *Nosema*, which chronically infects the digestive tract of *Apis mellifera*. Therefore, developing solutions to improve bee health including nosemosis is increasingly necessary, but still lacking. Here, we focused our investigations on the development of Matrix-assisted laser desorption/ionization imaging (MALDI) molecular mass fingerprint (MFP) and its integration with MALDI imaging mass spectrometry (MALDI IMS) to monitor which tissues are impacted by *Nosema ceranae*. MALDI IMS is a powerful technology used to investigate the spatio-temporal distribution of a large number of molecules throughout a body/tissue section. Here, we report the use of MALDI IMS to follow the molecular impact of an experimental infection of honeybees with the microsporidia *N. ceranae*. We analyzed representative MFP of selected tissues. This was followed by MALDI IMS workflows optimization including specimen embedding and positioning as well as washing and matrix application. We recorded the local distribution of peptides/proteins within different tissues from experimentally infected versus non-infected honeybees. As expected, a distinction in these molecular profiles between the two conditions was found in different anatomical sections of the gut tissue. More importantly, we observed differences in the molecular profiles of the brain, thoracic ganglia, hypopharyngeal gland, and hemolymph. We introduced MALDI IMS as an effective approach to monitor the impact of *N. ceranae* infection and applied this approach to a model of interaction between CBPV and the insecticide sulfoxaflor on *Apis mellifera*. This opens perspectives for the discovery of molecular changes in peptides/proteins markers that could contribute to a better understanding of the impact of stressors and toxicity on different tissues of honeybees.

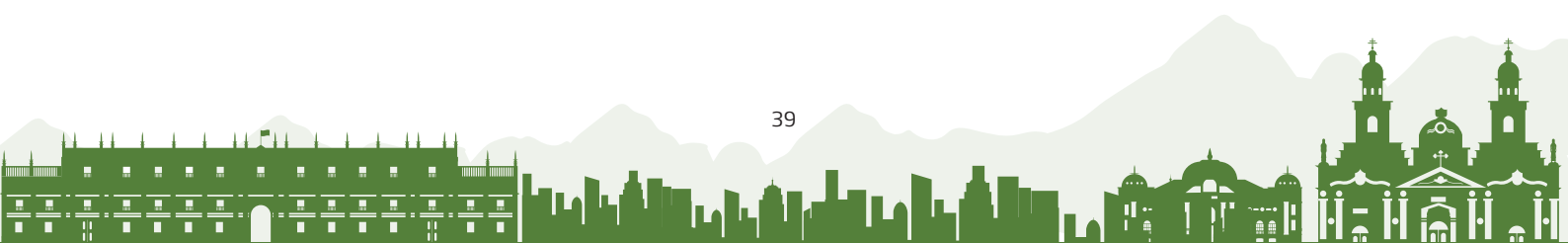
### OP-066

## Progress on the bee breeding program for low Varroa growth (LVG) in Canada

Ernesto Guzman-Novoa, Alvaro De La Mora, Nuria Morfin, Paul H Goodwin, Paul G Kelly, Berna Emsen

School of Environmental Sciences, University of Guelph, Guelph ON, Canada

One alternative to control Varroa destructor infestations in honey bee colonies is to breed Varroa-resistant strains of honey bees. We are currently conducting the fourth generation of a breeding program to select bees for low and high rates of *V. destructor* population growth (LVG and HVG, respectively). After three years of bidirectional selection, significant differences between the two genotypes were observed. LVG colonies had *V. destructor* population increases over the summer of 3 fold compared to 6 fold for HVG colonies. Additionally, LVG colonies had significantly lower mite infestation rates and 60% more mutilated mites compared to HVG colonies. Hemocyte concentration in the hemolymph and grooming responses were significantly higher in LVG bees than in HVG bees. No differences for hygienic behavior were found between the two genotypes. Winter mortality rates of colonies were significantly lower for the LVG genotype than for the HVG genotype in the first two generations but not in the third one. The results of this study indicate that selection for LVG may result in colonies with lower *V. destructor* infestation rates, better immune state and possibly higher colony winter survivorship. Grooming behavior seems to be an important mechanisms contributing to the observed resistance against Varroa. Future work will focus on conducting molecular analyses of the genotypes to identify candidate genes associated with resistance to *V. destructor* that could potentially be used for marker-assisted selection of mite-resistant honey bee strains.





### OP-067

## Transcriptomic analysis of the honey bee (*Apis mellifera*) queen brain reveals that gene expression is affected by pesticide exposure during development

Juliana Rangel, Myra Dickey, Elizabeth M Walsh, Tonya F Shepherd, Raul F Medina, Aaron Tarone

Department of Entomology, Texas A&M University, College Station, TX, USA

Honey bees (*Apis mellifera*) play a pivotal role in agricultural production worldwide, primarily through the provision of pollination services. But despite their importance, honey bee health continues to be threatened by many factors, including parasitization by the mite *Varroa destructor*, poor queen quality, and pesticide exposure. Accumulation of pesticides in the hive's comb matrix over time inevitably leads to the exposure of developing brood, including queens, to wax contaminated with multiple compounds. Here, we characterized the brain transcriptome of queens that were reared in wax contaminated with pesticides commonly found in commercial beekeeping operations including either (a) a combination of 204,000 ppb of tau-fluvalinate and 91,900 ppb of coumaphos ("FC" group), (b) a combination of 9,800 ppb of chlorpyrifos and 53,700 ppb of chlorothalonil ("CC" group), or (c) 43,000 ppb of amitraz ("A" group). Control queens were reared in pesticide-free wax. Adult queens were allowed to mate naturally before being dissected. RNA isolated from brain tissue from three individuals per treatment group was sequenced using three technical replicates per queen. Using a cutoff log<sub>2</sub> fold-change value of 1.5, we identified 247 differentially expressed genes (DEGs) in the FC group, 244 in the CC treatment group, and 668 in the A group, when comparing each group to the control. This is the first study to examine the sublethal effects of pesticides commonly found in wax (particularly amitraz) on the queen's brain transcriptome. Future studies should further explore the relationship between our molecular findings and the queen's behavior and physiology.

### OP-068

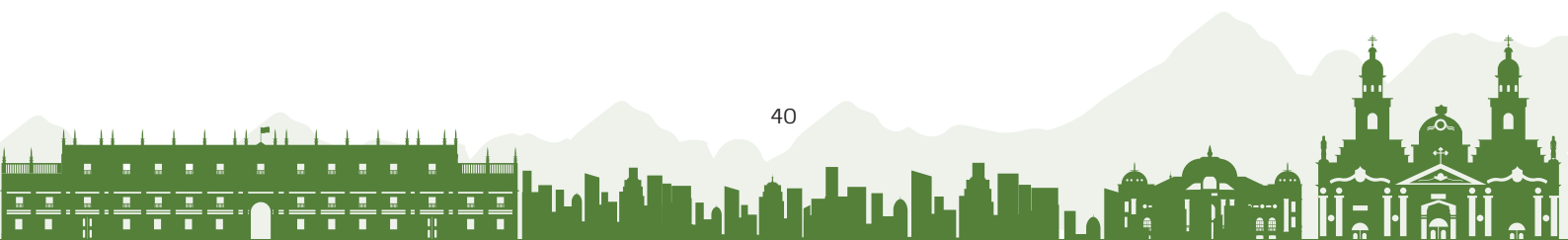
## Unraveling honey bee's virome from varroa survivor and susceptible colonies in Uruguay

Daniela Arredondo<sup>1</sup>, Sofia Grecco<sup>2</sup>, Yanina Panzera<sup>2</sup>, Pablo Zunino<sup>1</sup>, Karina Antúnez<sup>1</sup>

<sup>1</sup>Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, IIBCE, MEC, Montevideo, Uruguay

<sup>2</sup>Sección Genética Evolutiva, Departamento de Biología Animal, Facultad de Ciencias, UdelaR, Montevideo, Uruguay.

*Varroa destructor* is the main biotic threat to *Apis mellifera*, it is a vector of various viruses, and in several cases, it could be lethal if colonies are not treated with acaricides. However, around the world some honey bee's populations survive naturally to mite parasitization without treatments. In the Norwest of Uruguay, there is a population with these characteristics. This phenomenon is associated with the hygienic behavior of these bees, but it could also be associated to the presence of different viral communities. This work aims to characterize the viruses in survivor and susceptible *A. mellifera* colonies. To accomplish this objective, we used nurse samples collected from surviving and susceptible colonies in spring and fall. We used a viral particle enrichment protocol, massive sequencing and bioinformatic analysis to unravel the honey bee viromes. We obtained reads from all the samples, in spring we did not find significant differences between the number of reads from susceptible and survivor colonies, although we found a strong presence of reads mapping *Varroa destructor* virus, also known as Deformed wing virus type B in the susceptible colonies. In the fall, the susceptible colonies show significative more reads than survivor colonies. In this work we also obtained the complete genome sequences from Acute Bee Paralysis Virus, Black Queen Cell Virus, Deformed Wing Virus and Sacbrood Virus for the first time in our country. In addition, we also obtained the complete genome sequences from viruses that had not yet been detected in our country; this is the case of the Lake Sinai virus and the *Apis mellifera* filamentous virus. We continue studying these viral profiles of the surviving and susceptible colonies to improve our understanding of the complex interaction virus-varroa-honeybee.



### OP-069

#### The blueberries and the bees: assessing honey bee health stressors using proteomics

Rhonda Thygesen<sup>1</sup>, Leonard Foster<sup>1</sup>, Renata Moravcova<sup>1</sup>, Stephen F Pernal<sup>2</sup>, Marta M Guarna<sup>2</sup>, Shelley Hoover<sup>3</sup>, Rob Currie<sup>5</sup>, Amro Zayed<sup>4</sup>, Pierre Giovenazzo<sup>6</sup>, Mateus Pepinelli<sup>4</sup>, Ida M Conflitti<sup>4</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada

<sup>2</sup>Beaverlodge Research Farm, Agriculture and Agri-Food Canada, Beaverlodge, Canada

<sup>3</sup>Department of Biological Sciences, University of Lethbridge, Lethbridge, Canada

<sup>4</sup>Department of Biology, York University, Toronto, Canada

<sup>5</sup>Department of Entomology, University of Manitoba, Winnipeg, Canada

<sup>6</sup>Department of Biology, Université Laval Québec, Québec City, Canada

Honey bee *Apis mellifera* pollination is essential for British Columbia's (B.C.) top fruit export, highbush blueberry (HBB, *Vaccinium* sect. *Cyanococcus*), to ensure high fruit set. Recently, B.C. beekeepers have noticed a decrease in health and strength of their colonies after HBB pollination, leading some to avoid contracts and causing financial strain on HBB growers; however, the risk factors affecting honey bee health in HBB pollination are not yet well defined. Pesticides, pathogens, pests and parasites are all possible effectors of decreased bee health in HBB. The proteome is central to health, and its composition is likely to vary with health status. Proteomics allows for the comparative study of an organism's proteome in healthy versus diseased state so diagnosis and treatment is feasible. Additionally, proteomic research does not require a large sample size from a living hive to obtain quantitative data. This study combines field and lab work to deduce what the major determinants of bee health in HBB are by observing protein signature change in bees. Two field seasons (2020 and 2021) were used to correlate differences in the proteome of nurse bees before, during, and after HBB pollination, as well as outside of HBB areas as a control. Pesticides and pathogens were also included as variables. Cage trials of individual xenobiotic or pathogen stressors found in HBB were subsequently performed to validate proteomic changes in order to define the main causes of stress in HBB pollination. Specifically, pyrimethanil, a fungicide used on HBB crops, and deformed wing virus, a microorganism affecting adult honey bee wing formation, were tested alone and together on caged bees to investigate protein changes.

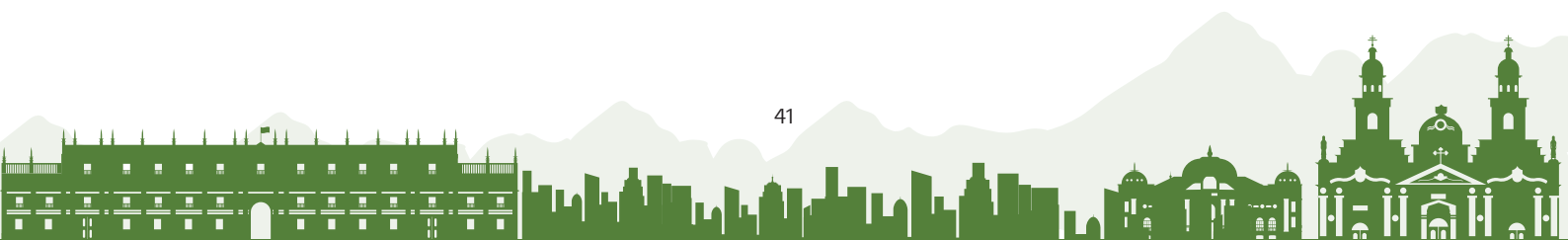
### OP-070

#### Fertility costs of cryptic viral infections in honey bee queens

Abigail Chapman, Alison McAfee, Leonard Foster

Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada

Poor quality queens are a persistent problem for beekeepers, consistently ranking as one of the top reasons for colony loss in Canada, but what causes queen failure remains poorly understood. Our results suggests that viral infections occurring naturally in the field are one factor among many that is compromising the reproductive success of honey bee queens. Many insects are subject to a trade-off between reproduction and immune activity, meaning that infections can have indirect impacts on fecundity, even in the absence of overt symptoms. We have found that failed (poor quality) queens sampled from beekeepers across British Columbia and Alberta in two independent surveys have higher levels of viral infection and smaller ovaries, despite an absence of viral symptoms. Queens experimentally infected in the laboratory also exhibited a reduction in ovary mass - evidence that virus infection may actually cause a reduction in queen reproductive output, rather than merely being correlated with it. We are now investigating how virus infection in a queen affects the eggs that she lays using proteomics, lipidomics, and metabolomics. We are also conducting a field-trial this summer to determine if experimentally infected queens exhibit symptoms similar to those that beekeepers observe as failure, the results of which we are excited to present. Our findings suggest that honey bee queens may be subject to a reproductive compromise, and that virus infection is a contributing factor to queen failure in beekeeping operations.



### OP-071

## Varroa destructor resistance to acaricides: An overview of the situation and a focus in France

**Benjamin POIROT<sup>1</sup>, Gabrielle Almecija<sup>1</sup>, Précillia Cochard<sup>1</sup>, Christelle SUPPO<sup>2</sup>, Veronique Duquesne<sup>3</sup>, Paulo Mielgo<sup>4</sup>, Max Watkins<sup>4</sup>, Joel Gonzalez-Cabrera<sup>4</sup>**

<sup>1</sup>APINOV, Research and Training Center, 10 rue Henri Bessemer, 17140 Lagord, France

<sup>2</sup>Institut de Recherche sur la Biologie de l'Insecte, UMR 7621, CNRS-Université de Tours, 37200 Tours, France

<sup>3</sup>French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Sophia Antipolis Laboratory, Bee Pathology Unit, Sophia Antipolis, France

<sup>4</sup>Vita Bee Health, House, London St., Basingstoke, Hants, RG21 7PG, UK

<sup>5</sup>Instituto de Biotecnología y Biomedicina BIOTECMED, Universitat de València, Dr. Moliner 50, 46100 Burjassot, Spain

Varroa destructor is a parasite of honeybees. It causes biological damage leading to the collapse of a colony in the absence of treatment. The acaricide tau-fluvalinate was widely used in the 1990s until varroa developed resistance, significantly reducing the effectiveness of treatments in some areas. Now, the most-used conventional acaricide substance is amitraz. Unfortunately, varroa has also developed resistance to that active ingredient. The lecture will focus on varroa resistance to these two important acaricides: tau-fluvalinate and amitraz.

First, a phenotypic sensitivity test was developed to detect the presence of resistant mites. Using sensitive reference populations, we selected the discriminant concentration method (LC90) to determine the proportion of resistant mites in a population. In France, 45% and 66% of honeybee colonies are moderately or highly resistant to tau-fluvalinate and amitraz, respectively. We investigated the mechanism of the resistance to amitraz detected in *V. destructor* mites from French apiaries. The amino acid substitution N87S in the Oct $\beta$ R was associated with amitraz treatment failures reported by beekeepers. Resistance to tau-fluvalinate was previously associated with the presence of mutations at position 925 of the voltage-gated sodium channel (VGSC). However, other mutations in the target-sites or other mechanisms of resistance may also be involved in explaining the survival of varroa mites in the phenotypic test.

Then, using a model, we analysed the impact of various parameters, including the presence of resistant varroa on the efficacy of Apivar<sup>®</sup> treatment (amitraz). A moderately resistant population will result in a loss of efficacy of 5-10%, while a highly resistant population will result in an efficacy of less than 78%. Highly resistant populations observed in the field confirmed these simulated model results. We also found that initial infestation, treatment period and treatment formulation influenced the efficacy of treatment.

The management of varroa by beekeepers must now include resistance management to limit the spread of resistant mites. In this context, we suggest that phenotypic or genotypic tests would be a good tool to advise policymakers.

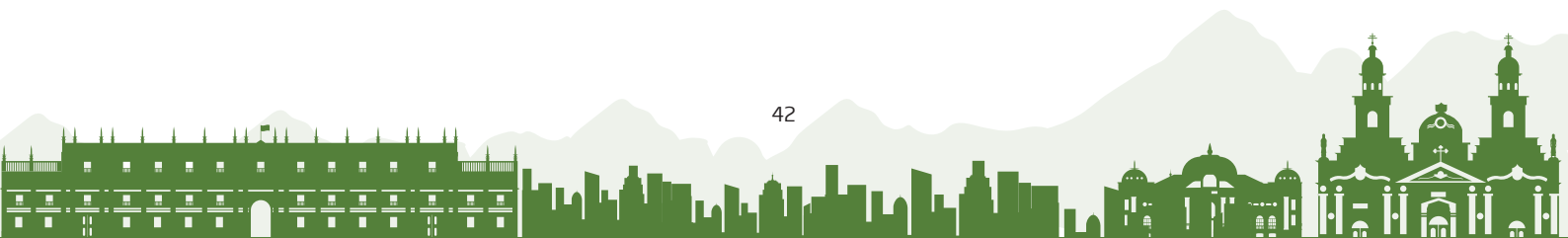
### OP-072

## A severe global alert on honey bee health: a recent unidentified devastating health phenomenon of honey bees of Iran

**Pegah Valizadeh, Nader Mashayekhi Sardouiee**

Honey Bee Research Department, Animal Science Research Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

Honey bees are constantly facing numerous pests and diseases all across the globe. Some of the pests and diseases of honey bees have been identified and well known for a long time. While others are either globally first time detected or new in a specific geographical location. The effect of different pests and diseases on the livelihood of an apiary and in general on the beekeeping industry of a country can be very diverse. Some of the pests and diseases would be with almost no detectable consequence, while some others are as devastating as ruining the entire apiary with the serious risk of an uncontrollable spreading. In Iran, since spring of 2022 a health defect has spread massively in apiaries all across the country, with a total area of 1,648,195 km<sup>2</sup> and very diverse climates. This phenomenon affects honey bees by killing the brood, causing an interruption of population growth, resulting in up to 100% colony destruction in some apiaries. The symptoms are somehow similar to European foulbrood (EFB) or American foulbrood (AFB). However, according to the microbiological tests it seems that the condition is independent from the presence or absence of the two bacterial diseases. In the last few years, the health defect has been scarcely detected in some apiaries of Iran; however, up until this last year it used to be completely negligible with no serious damage. Migratory system of the Iranian beekeeping as well the critical misuse of chemical drugs, antibiotics in specific, seem to intensify the condition immensely. The causing factor of the phenomenon is still unidentified. It could be a first time detected factor such as a virus, a bacterium, a fungus, a new pest, or conversely the health situation could be an integrated result of a network of already recognized factors and conditions. Unless the causing factor is identified, no sustainable treatment and prevention can be applied. At this point, it appears that the health phenomenon is still limited to the apiaries inside the borders of Iran. Nevertheless, the risk of its global spread is too serious to ignore. An international collaboration is demanded.



### OP-073

## Range Adjustment of Saturation Concentration of Formic acid-gel product "Mite-K" for Field Application

Jin Kyu Park, Jeong Dong Han, Chang Ki Ahn  
BEESENBIO CO., LTD.

Formic acids are eco-friendly substances that do not cause resistance to bee-mites, but when commercialized, both bees and beekeepers could be exposed directly to the formic acid, making them difficult to handle. In this study, we manufactured a chamber (225L) for measuring the vaporizing concentration before field application of the formic acid-gel product "Mite-K" according to time and the internal environmental changes (temperature, humidity, and exhaust speed etc.). The range adjustment of vaporized concentration of formic acids was established which can most efficiently eradicate honeybee mites by "Mite-K". We investigated that the compositions of the gel ingredients directly affect and make changes of vaporized patterns over time. To eliminate mites while protecting honeybees from being stressed by formic acid, and to observe which level of formic acid can make eradication of the parasitic mites even by invading into the larvae in the honeybee rooms more effectively. We needed to control the saturation concentration of formic acids within a certain range for a certain range of time, considering the factors that affect bees (wind direction, temperature and humidity and the number of bees and frames of comb inside the hive etc.). Considering these factors, the range of formic acid saturation concentration for efficient removal of honeybee mites were estimated to be 50ppm to 250ppm, but this was mostly dependent on the self-evaporation control ability of formic acid-containing gel and the temperature, humidity, and exhausted velocity from the beehive. When the temperature inside the chamber was 20 to 33 °C, humidity was 26 to 45%, and exhausted velocity was 60 to 80cc/min, the maximum concentration (Max. 559ppm) of vaporized formic acid from Mite-K could be adjusted to 230ppm by changing the composition of the gel ingredients, and the retention time of the valid concentration was about 15 hours. If the internal temperature rises above 35 °C and/or the humidity rises by more than 60%, it is necessary to use another product (Mite-K plus) which have a different gelation composition ratio for the effective removal of honeybee mites.

Key words: Formic acid-gel product (Mite-K/Mite-K plus), Adjustment of Saturated concentration, Honeybee mites  
Corresponding author(jdhan7415@gmail.com), \*presentation author

### OP-074

## Different bottom boards in honeybee hives for the control of Varroa destructor

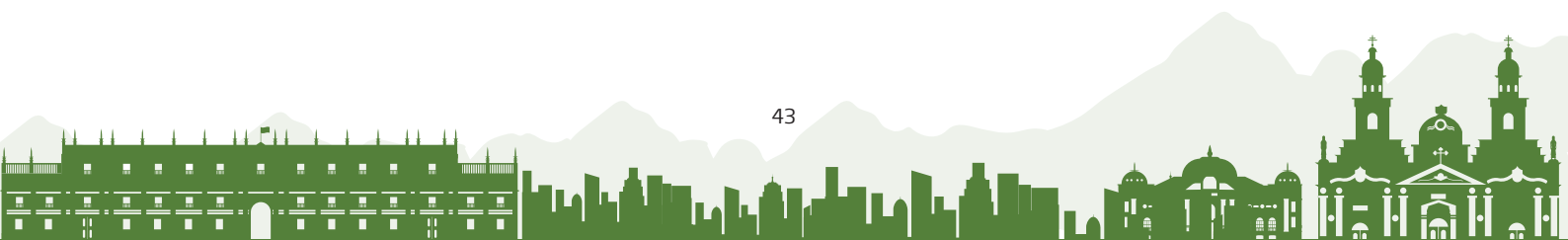
Pablo Juri<sup>1</sup>, Enrique Nogueira<sup>1</sup>, José Anzola<sup>1</sup>, Valentina Rodríguez Batista<sup>1</sup>, Belén Branchiccela<sup>3</sup>, Ciro Invernizzi<sup>2</sup>

<sup>1</sup>Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay

<sup>2</sup>Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

<sup>3</sup>Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay

Varroa destructor is the main pest of honey bees worldwide. This ectoparasitic mite causes direct damage to bees. In addition, it is the vector of numerous viruses. Currently, integrated pest management is considered the best strategy to minimize the use of acaricides. Designed bottom boards that can slow the growth of the mite population by removing them from the hive or preventing them from returning and reinfesting the colony could be used. The aim of this study was to compare the effect of three different bottom boards in colonies strength and infection level of Varroa destructor over time. An apiary of 55 colonies was randomly divided into five groups: 1) Tubular bottom board, 2) Mesh bottom board, 3) Sticky bottom board, 4) Permanent Varroa treatment with conventional bottom board (positive control), and 5) Untreated colonies with conventional bottom board (negative control). Infection level of Varroa destructor in nurse bees was determined and colony strength (adult and brood population) was estimated in April, June, August, October and December 2021. Significant differences were observed in the sticky bottom board group respect to the negative control in December sampling, limiting the growth of Varroa populations. Mesh bottom board group, presented similar results to the untreated group during the first part of the study. However, by the end of the study, the Varroa population did not increase in colonies, this mesh bottom board suggests that it may be efficient to not increase damage when there are high rates of infestation. No significant differences were found between tubular bottom boards and negative control. Finally, no differences between groups are observed in the strength of the colony, both in adult and brood population. In conclusion, use of an adhesive in the bottom board to retain the detached Varroas can be a practice that contributes significantly to the varroosis control and reduce the applications of acaricides.



### OP-075

## Prevalence and genetics of amitraz resistance in *Varroa destructor* from the US

Frank Rinkevich

USDA-ARS Honey Bee Breeding, Genetics, and Physiology Lab, Baton Rouge, Louisiana, USA

*Varroa destructor* is the most significant and ubiquitous threat to honey bee colony health around the world. Beekeepers often rely on miticide application to manage *Varroa* populations due to its ease of use and scalability. However, overuse and overreliance on miticides may cause the development of resistance. Amitraz resistance in *Varroa* is a rising concern to beekeepers across the world. Four years of monitoring efforts in the US have shown amitraz resistant *Varroa* populations with a trend towards more frequent detection of resistant populations. A mutation in the  $\beta 2$  octopamine receptor was confirmed to be strongly associated with the amitraz resistance phenotype. However, genetic testing suggested the resistance bioassay used in the field underestimated amitraz resistance due to some individuals with the resistant genotype appearing phenotypically susceptible. Lab experiments showed that high temperatures can induce *Varroa* to appear phenotypically susceptible regardless of genotype. Low temperatures showed reduced efficacy in the resistance test and these findings were corroborated by reduced amitraz sensitivity at low temperatures in bioassays with technical grade amitraz. Genomic sequencing showed genetic variation in *Varroa* collected during the monitoring program. Identification of genomic regions associated with amitraz resistance as well as population structure and kinship analyses are ongoing research projects. A survey of amitraz use patterns by beekeepers is being conducted to understand the conditions that may create amitraz resistant *Varroa* populations. The data generated from resistance monitoring network and genome analyses serve as the foundation for the synthesis of holistic and effective amitraz resistance management strategies in *Varroa*.

### OP-076

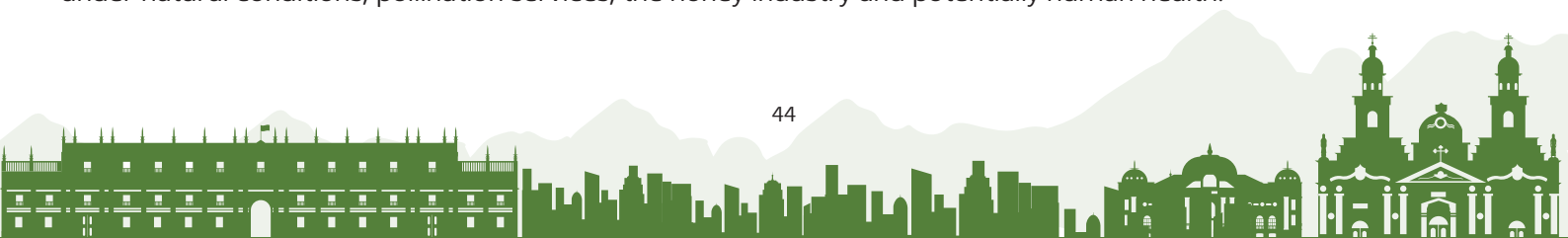
## Biomolecular and behavioural responses of the European honey bee ("*Apis mellifera*") to sublethal perfluorooctane sulfonate exposure concentrations

Carolyn Anne Sonter<sup>1</sup>, Romina Rader<sup>1</sup>, Manisha Shakya<sup>1</sup>, Matthew Tighe<sup>1</sup>, Gavin Stevenson<sup>2</sup>, Susan Caroline Wilson<sup>1</sup>

<sup>1</sup>Environmental and Rural Science, University of New England, Armidale, NSW, Australia

<sup>2</sup>National Measurement Institute, Ryde, Sydney, NSW, Australia

Bees provide pollination services to managed and wild ecosystems but are threatened globally by multiple stressors, including pesticides. Perfluorooctane sulfonate (PFOS), a persistent contaminant that accumulates and biomagnifies up the food chain, is a breakdown product of the widely used pesticide sulfluramid, which is used to control leaf cutting ants in eucalyptus forestry. Guidelines for ecological receptors to PFOS exposure do not currently exist due to insufficient available data. In this exposure effect study, small whole colonies of "*Apis mellifera*" (2500 bees) were exposed to PFOS using a purpose-built cage system over a five-week period. Perfluorooctane sulfonate exposure concentrations ranging from 5 to 20  $\mu\text{g L}^{-1}$  were provided to bees in sugar syrup. This concentration range was lower than concentrations reported lethal to bees, but also spanned environmental water concentrations reported. A range of biomolecular and behavioural responses were monitored. Bee head tissue was analysed for protein content, lipid peroxidation, protein carbonyl and for proteomic changes due to oxidative stress. Bee tissue, honey and faecal matter were analysed to determine PFOS concentration using isotope dilution combined with Liquid Chromatography Tandem Mass Spectrometry adapted for bee and honey matrix analysis. Brood development and newly emerged bee weight decreased significantly at  $\geq 5 \mu\text{g L}^{-1}$  and gastric health was negatively impacted with any PFOS exposure compared to the controls. Consumption and storage of sugar syrup as honey decreased with increasing PFOS concentration and was significantly lower than the control at the lowest exposure concentration of  $\geq 5 \mu\text{g L}^{-1}$ . Perfluorooctane sulfonate was detected in adult and newly emerged bee tissue with a mean bioaccumulation factor (BAF) of 1.72 and 0.69, respectively. Perfluorooctane sulfonate was also identified in honey and faeces collected from the cage hives. These findings provide clear evidence that PFOS exposure in such low concentrations adversely affects honey bee colonies, causing biomolecular and behavioural changes in bees and may transfer to honey. Our study contributes to the development of ecological protection guidelines and has implications for exposed populations under natural conditions, pollination services, the honey industry and potentially human health.





### OP-077

## Always Arid: Lessons from Atacama Desert Beekeeping in the Face of a Changing Climate

Mónica Meza Aliaga<sup>1</sup>, Manuel Prieto<sup>1</sup>, Monserrat Uribe<sup>1</sup>, Francisco Mayol<sup>1</sup>, Maritza Paicho Hidalgo<sup>1</sup>, Karen Yáñez<sup>2</sup>, Ariel Muñoz Navarro<sup>3</sup>

<sup>1</sup>Department of Historical and Geographical Sciences, University of Tarapacá, Chile

<sup>2</sup>Biotechnology Center, Federico Santa María Technical University, Chile

<sup>3</sup>Institute of Geography, Pontifical Catholic University of Valparaíso, Chile

Since the beginning of beekeeping in the Atacama Desert (AD), beekeepers have developed strategies, practices, and techniques to cope with the arid landscape. These conventional strategies and practices hold valuable lessons for adaptation to the challenges of climate change (CC) in areas that are beginning to desertify.

The AD is a highly vulnerable region to the adverse effects of CC due to its geographical location, while the challenge of honey production in the central-southern region of Chile lies mainly in the fight against desertification. Consequently, desert beekeeping offers lessons and a unique opportunity to study traditional adaptation strategies that have proven effective in tackling problems related to climate change.

To study how CC affects beekeeping activities, the social and natural dimensions of the site must be considered. Semi-structured interviews and participant observation were conducted to document, among other things, various honey production strategies, such as the alternating cultivation method of nectar-producing flora, "weed" irrigation systems, nutritional supplements, waterers, and nest size reduction, among others. Despite its success, beekeeping in the desert faces additional challenges arising from the intensification of known threats and new ones. Among the former, we mention the decrease in precipitation, extreme weather events, strong winds, and temperature increase. Among the latter, we have the increase in extractive industries, changes in land use, extreme protection of private property, particulate matter, changes in land use, and pesticide use.

Therefore, it is essential to continue researching and perfecting adaptation strategies in the AD in order to promote the development of sustainable and resilient beekeeping production, capable of facing the adversities of climate change and increasingly intense external pressures. The application of these techniques will ensure the viability and survival of beekeeping in the AD and other regions affected by climate change, as well as contribute to the maintenance of biodiversity and ecosystems.

### OP-078

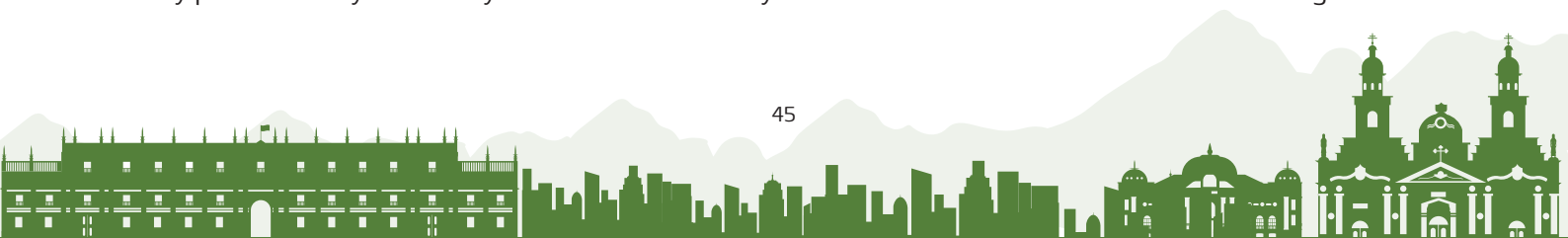
## Secret sounds of bees: The use of vibroacoustics and Hidden Markov Models for non-invasive honey bee colony health monitoring

Carol Fassbinder-Orth<sup>1</sup>, Amara Orth<sup>2</sup>

<sup>1</sup>Creighton University

<sup>2</sup>Stanford University

Non-invasive health monitoring technology is a rapidly growing area of human and animal health alike. For honey bees, one under-explored metric that can be used for colony health monitoring is vibroacoustics. Vibroacoustics refers to sounds and vibrations that are emitted by bees in response to stimuli and may be essential to understand more about honeybee behavior and health. Hidden Markov Models (HMMs) are sound processing algorithms that have been used to study bioacoustic signals in a wide variety of organisms. HMMs process signals in a statistical manner and can characterize highly complex and variable signals. In this project, honey bee bioacoustic signals were collected using piezoelectric microphones attached to the top bars of 110 colonies monthly for 1 year in Iowa, USA, Canterbury, New Zealand, and Chatham Islands, New Zealand. Over 1200 sound files and 40 hours of sound were collected and labeled using Audacity according to several quantifiable colony states: queenright, queenless, virgin queen, robbers, wax moths, varroa mites, honey flow, and rapid population decline. These labeled sound files were then used to train the HMMs through a MATLAB Hidden Markov Model Toolkit (MATLABHTK). The model successfully recognized all colony states with a range of accuracy. Queenless states were detected with up to 100% accuracy, while honey flow and robbing had accuracy levels generally less than 80%. These results indicate that bioacoustic signals and HMMs can accurately predict honeybee colony health states and may be a useful tool in beehive health monitoring.





### OP-079

#### Pathogen dynamics in a varroa-free world: a comparison of honey bee pathogens in the Chatham Islands, New Zealand and mainland New Zealand

Carol Fassbinder-Orth<sup>1</sup>, Peter Dearden<sup>2</sup>

<sup>1</sup>Biology Department, Creighton University, Omaha, NE, USA

<sup>2</sup>Biochemistry Department, University of Otago, Dunedin, New Zealand

With the recent discovery of *Varroa destructor* (varroa mites) in honey bee colonies in New South Wales, Australia, there remain only a few inhabited places on earth that are certified varroa-free. One such location is the remote Chatham Islands, an archipelago 800 km east of New Zealand. Although varroa mites were detected on mainland New Zealand over 20 years ago, the honey bee colonies of the Chatham Islands remain varroa-free and provide us with an ideal opportunity to study the impacts of varroa mites on honey bee health and disease. In this study, nest worker bees were collected monthly from commercial honey bee colonies on the south island of New Zealand (n=50) and the Chatham Islands (n=10). The following pathogens were quantified using droplet digital PCR (ddPCR): *Nosema ceranae*, *Paenibacillus larvae*, Chronic bee paralysis virus, Deformed wing virus, Kashmir bee virus, Lake Sinai viruses 1,2, and 4, and Sacbrood virus. Levels of expression of vitellogenin and also juvenile hormone (JH) acid O-methyltransferase (JHAMT) were analyzed for their potential utility as colony health status markers. Lastly, varroa mite levels were quantified in the mainland New Zealand colonies using monthly alcohol washes. *P. larvae* and Lake Sinai virus 4 were not found in any colonies studied. All other pathogens were detected at some point throughout the year in the South Island bees. The number of different viruses detected monthly in Chatham Islands was significantly lower than in the mainland New Zealand bees. Also, colonies infested with varroa mites exhibited lower vitellogenin levels than colonies without detectable varroa. Vitellogenin is an important hormone involved in immune function, metabolism, and bee development, and our results suggest that it is a useful marker for varroa's impact on colony health in this study. In conclusion, studying pathogen dynamics and colony health markers in varroa-free areas provides us with an invaluable resource to study the impacts of varroa on colony health, and anti-importation regulations need to be strengthened in these areas to ensure that these last remote areas on earth remain varroa-free.

### OP-080

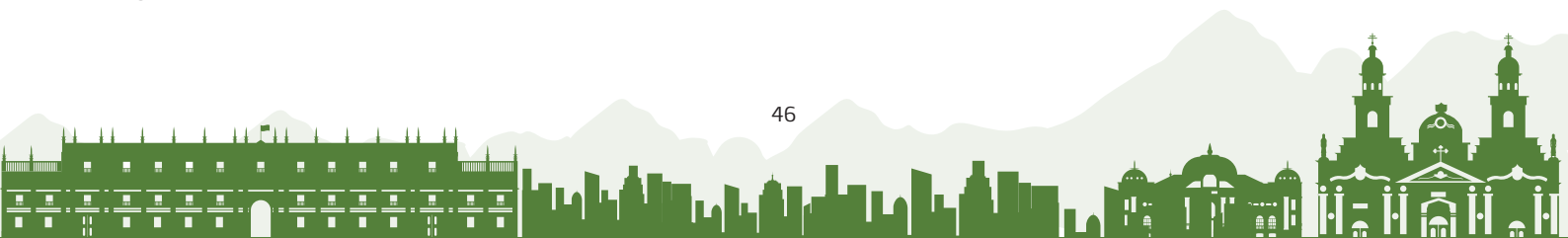
#### Modeling floral diversity suggests a dilution effect for RNA viruses in transmission between honeybees and bumblebees

Alex Burnham<sup>1</sup>, Samantha Alger<sup>2</sup>

<sup>1</sup>Department of Biology, University of Vermont, Burlington, Vermont, United States of America

<sup>2</sup>Plant and Soil Science Department, University of Vermont, Burlington, Vermont, United States of America

Both managed and wild bees face a growing number of threats including the spread of RNA viruses between and within bee species. For example, RNA viruses, as well as some other shared pathogens, have been shown to be transmitted between honeybees and bumblebees through the use of shared flowers. As a bridge of transmission, understanding the roles that floral abundance, diversity and composition play in modifying transmission rates is an important step in reducing transmission on the landscape. Flower species are known to harbor viruses differentially. These nonuniform distributions coupled with bee's nonrandom foraging strategies (such as floral constancy) may provide a mechanism for flowers to influence transmission through dilution or amplification effects. In this study, we used an agent-based modeling approach to test how bee and floral abundance, bee foraging strategies, and floral diversity influenced transmission of a DWV-like virus. We found that transmission was reduced when modeled in diverse landscapes as compared to monocultures providing evidence for a dilution effect in this system. Increased floral abundance also reduced transmission in both bumblebees and honeybees by reducing the likelihood of bees of both genera visiting the same flower. Finally, when bees exhibited higher levels of floral constancy, transmission was further reduced. These results indicate that disease reduction in bee species could be accomplished by managing floral landscapes. By increasing floral diversity and abundance, we may not only improve forage and habitat for bees and other animal species, but also decrease virus transmission. Future empirical studies that build on our results should test this theory in the field and aim to provide guidelines surrounding flower planting and management strategies.



### OP-081

## The complex reality of the beekeeper's efforts to keep healthy honey bees. Communication and applied research on the agenda for the COLOSS B-RAP group

Lotta Fabricius Kristiansen<sup>1</sup>, Linde Morawetz<sup>2</sup>

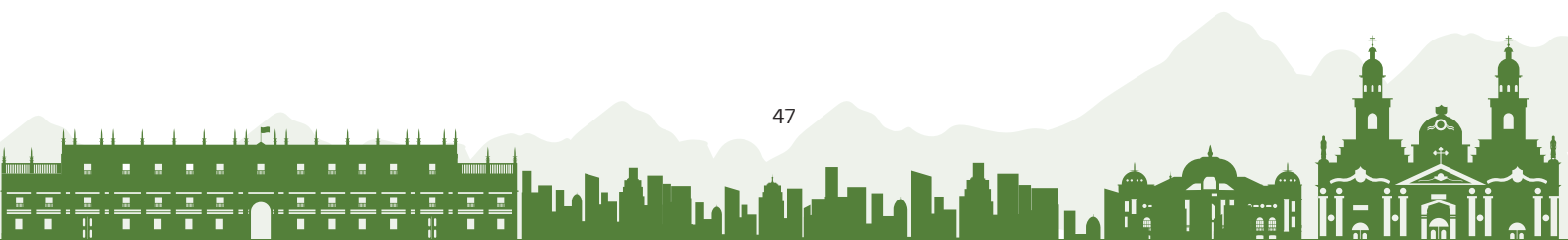
<sup>1</sup>Swedish University of Agricultural Sciences, National Competence Center for Advisory Services, Department of People and Society, Alnarp, Sweden.

<sup>2</sup>Department for Apiculture and Bee Protection, Institute for Seed and Propagating Material, Phytosanitary Service and Apiculture, Division for Food Security, Austrian Agency for Health and Food Safety Ltd., Vienna, Austria

The main mission of the COLOSS honey bee research association is to improve the well-being of honey bees. A way to obtain this is by using the power of collaboration between the members, who mainly are researchers in different academic fields, veterinarians and advisors. COLOSS has 12 organized groups working with different topics related to honey bee health. One of the groups, B-RAP (Bridging Research and Practice), was established with the specific intention to connect science and beekeeping through the work of beekeeping advisory services.

Beekeepers are confronted with a large amount of information through a variety of sources such as internet, beekeeping literature and networks of co-beekeepers. It is hard for beekeepers to know who and what is credible and trustworthy, as information is often inconsistent and contradictory. Finding the "correct" information becomes even more complex when considering that the problems are multi-scalar, and solutions often need to be based on local conditions. Local adaptation and sharing of context-specific research results and practical experience are necessary, and generally facilitated through beekeeping extension activities. This adapted knowledge needs to be accessible to beekeepers in an user-friendly form – ideally through a network of various supporting structures focusing on different aspects of beekeeping. These various sources to knowledge and support for innovation in beekeeping is defined as a Beekeeping Knowledge and Innovation System (B-KIS). How can the B-KIS be analyzed, developed and improved in order to obtain healthier honey bees?

There are significant differences between different countries in terms of stakeholder involvement, existing networks, and funding. Such differences can be made visible by using the B-KIS model, which allows a quick structured overview of the main actors within the beekeeping sector and their relationships with each other. In the presentation this tool will be demonstrated. The uniqueness of different national governance, cultural and practice-based structures require different approaches. Some components may be universal, others are much more specific, demanding context precise solutions to be able to strengthen advisory services. Best fit and best practice examples help beekeepers in the different countries to find their own solution / model / extension structures.



### OP-082

## Assessing the efficacy of in-field and laboratory methods for the detection of *Tropilaelaps* spp

Maggie C Gill<sup>1</sup>, Bajaree Chuttong<sup>2</sup>, Paul Davies<sup>1</sup>, Dan Etheridge<sup>1</sup>, Lakkhika Panyaraksa<sup>2</sup>, George Tonge<sup>1</sup>, Victoria Tomkies<sup>3</sup>, Giles E Budge<sup>4</sup>

<sup>1</sup>National Bee Unit, Animal Plant Health Agency, Room 11G03, York Biotech Campus, Sand Hutton, York, YO41 1LZ, UK.

<sup>2</sup>Meliponini and Apini Research Laboratory, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, 50200, Thailand.

<sup>3</sup>Fera Science Ltd, York Biotech Campus, Sand Hutton, York, YO41 1LZ, UK.

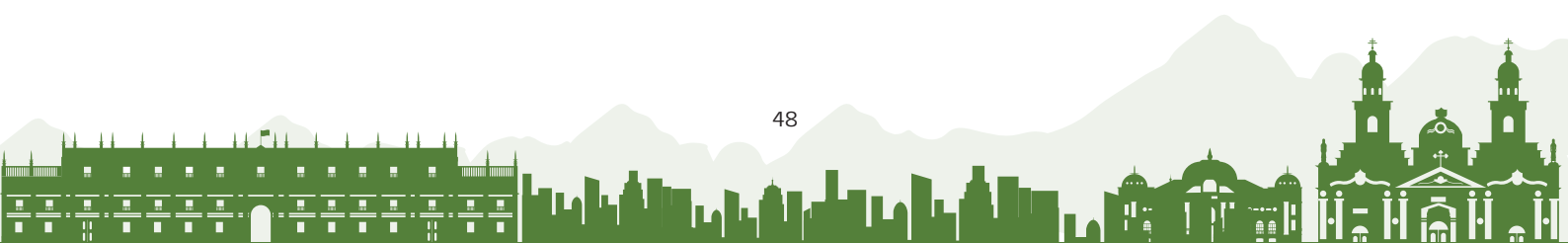
<sup>4</sup>Modelling Evidence and Policy Research Group, School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, Tyne, and Wear, NE1 7RU, UK

*Tropilaelaps* mites are native brood parasites of Asian honey bees that subsequently spread to *Apis mellifera* after this species was introduced into Asia. Infestations of *A. mellifera* by *Tropilaelaps mercedesae* occur in regions well beyond the distribution of their native Asian honey bee hosts. In addition, ever increasing global trade provides new transmission routes making these mites an important emerging global threat to *A. mellifera*. *Tropilaelaps* spp. are deemed statutory notifiable pests in England and Wales. The National Bee Unit (NBU) and Fera Science Ltd are responsible for the deployment of a national scheme for the surveillance and diagnosis of *Tropilaelaps* spp. in honey bee (*A. mellifera*) colonies using standard operating procedures (SOPs). This work sought to test the sensitivity and practicability of existing national SOPs against new methods for the detection of *Tropilaelaps* spp. mites in honey bee colonies.

In total, 60 *A. mellifera* colonies in Chiang Mai province, Thailand were monitored for the level of *T. mercedesae* using existing SOPs to include brood uncapping, floor debris sampling, comb bumping and the use of sticky floor inserts. Additional novel methods, traditionally used for *Varroa* monitoring were also trialled to include alcohol wash, CO<sub>2</sub> sampling and icing sugar roll of approximately 300 adult honey bees.

Our results indicated that methods developed for *Varroa* monitoring offered a significant improvement in mite detection compared to the methods detailed in existing SOPs. Brood uncapping and sugar roll were the most reliable detection methods, with the latter method having the advantage of being non-destructive. Comb bumping, alcohol washing and CO<sub>2</sub> were not reliable for the detection of *T. mercedesae*.

The study highlights the importance of using 'real life' field trials when assessing method efficacy of the detection of exotic pests. Our results have led to changes in the implementation of *Tropilaelaps* spp. surveillance policy in England and Wales, providing more robust methods of detection for these damaging invasive mites.



### OP-083

#### Bee-mediated monitoring of pesticides, pathogens, and emerging threats

M Marta Guarna<sup>1</sup>, Morgan M Cunningham<sup>1</sup>, Lan Tran<sup>1</sup>, Rodrigo Ortega Polo<sup>2</sup>, Stephen F Pernal<sup>3</sup>, Jonathan S Griffiths<sup>4</sup>, María Murcia Morales<sup>5</sup>, Amadeo R Fernández Alba<sup>5</sup>, Guillaume J Bilodeau<sup>6</sup>, Michael E Rott<sup>7</sup>

<sup>1</sup>Department of Agriculture and Agri-Food, Science and Technology, Gov. of Canada, Beaverlodge, AB, Canada, Department of Computer Science, University of Victoria, Victoria, BC, Canada

<sup>2</sup>Department of Agriculture and Agri-Food, Science and Technology, Gov. of Canada, Lethbridge, AB, Canada

<sup>3</sup>Department of Agriculture and Agri-Food, Science and Technology, Gov. of Canada, Beaverlodge, AB, Canada

<sup>4</sup>Department of Agriculture and Agri-Food, Science and Technology, Gov. of Canada, London, ON, Canada

<sup>5</sup>Department of Analytical Chemistry, University of Almeria, Almeria, Spain

<sup>6</sup>Canadian Food Inspection Agency, Ottawa, ON, Canada

<sup>7</sup>Canadian Food Inspection Agency, Sidney, BC, Canada

The European honey bee (*Apis mellifera*) plays a crucial role in many agricultural systems because of their global pollination services. In addition, honey bee colonies can serve as monitors of the surrounding environment. Bees are exposed to pathogens, pesticides and other pollutants during foraging, carrying them to the hive where they can be detected and quantified. We will present results of testing of plant pathogens and pesticides in hive matrices, and discuss recent developments including a non-invasive sampler, the "APIStrip" (Absorbing Pesticides In-hive Strips) developed in the framework of the INSIGNIA project, to monitor for agrochemical exposure. In addition, we will discuss the potential of using honey bee colonies for the detection of emerging threats such as invasive species and antimicrobial resistance (AMR) genes. For the identification of AMR genes, we used metagenomic sequencing of the honey bee gut microbiome. We identified tetracycline resistance genes as expected due to the widespread use of oxytetracycline in apiculture. Interestingly, we also identified AMR genes for products not used to treat honey bee colonies, indicating the utility of bee-based environmental monitoring. Monitoring AMR genes in apiaries and their surrounding environment can guide decisions towards limited and informed antibiotic use. We will also report our recent results analyzing bees and bee-collected pollen from pollinating colonies in blueberry, apple and cherry fields in British Columbia, Canada. We established the presence of plant viruses, fungi, and bacteria using high-throughput sequencing, including twenty-nine unique plant viral species in two blueberry production systems. Although additional research is needed, bee-based surveillance of plant pathogens, agrochemicals, and AMR genes has the potential to be an effective tool in environmental monitoring programs, crucial for protecting human, agriculture, and overall ecosystem health.

### OP-084

#### Factors leading to the loss of honeybee health (*Apis mellifera* L.) in managed colonies from Latin America: A holistic approach

Marnix Doorn<sup>1</sup>, María Mayda Verde Jiménez<sup>1</sup>, Leslie Vallejos<sup>1</sup>, Verónica Rachel Olate Olave<sup>5</sup>, Rafael Rolando Calderón Fallas<sup>2</sup>, Johan Wilhelm Van Veen<sup>2</sup>, Andrea Sánchez Chaparral<sup>4</sup>, Leonel Pérez Raymonda<sup>3</sup>, María Carla Cortese<sup>3</sup>

<sup>1</sup>Innovation & Extension, UC Davis Life Science Innovation Center, Santiago, Chile

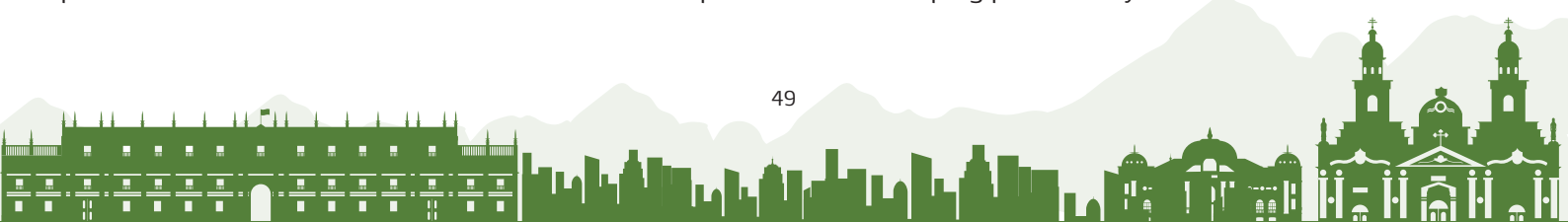
<sup>2</sup>Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, Heredia, Costa Rica

<sup>3</sup>Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Casilda, Argentina

<sup>4</sup>Corporación Universitaria UNICOMFACAUCA, Popayan, Colombia

<sup>5</sup>Instituto de Investigación Interdisciplinaria, Universidad de Talca, Talca, Chile

Managed honeybees (*Apis mellifera* L.) play an important role as pollinators, supporting most of the global plant diversity, associated organisms, and global agriculture. The health and nutritional condition of honeybee colonies depends on an important part of management practices, and it is influenced by multiple factors. Available information about bee health in Latin America is mainly focused on colony losses, diseases caused by pathogens, and the effect of pesticides, but relatively little information is centered on other variables, especially, those factors that contribute to visualizing health management with a "one health" approach. To identify some of the multiple factors related to honeybee health in Latin America, the project "Bee Health 2020 LatAm" (2017 to 2020) was implemented in specific regions of Chile, Colombia, Argentina, and Costa Rica. Field data were collected through a survey, considering different aspects related to management practices, productivity, clinical observations related to diseases, and the presence of sanitary gaps in the apiaries, among others. In addition, infestation rates by *Varroa* sp. mites were measured, and colony strength was evaluated as well. In total, 262 apiaries from the mentioned countries were studied, and around 1500 colonies (Langstroth) from different ecosystems and randomly selected, were monitored. In conclusion, some of the most relevant factors leading to the loss of honeybee health were: a) Insufficient public policies ensuring the professionalization of beekeeping activities on a scientific basis, b) Scarce transfer of knowledge from the academic to the productive sector, c) Presence of sanitary gaps, including the unsatisfactory disinfection of beekeeping materials, poor and inadequate bee queen replacement, deficiencies in feeding and colony strength, among others. The results of the study could contribute to novel approaches related to public policies and the improvement of local realities with economic impact. Territorial planning inherent to health management with a holistic and preventive approach, is presented as a viable solution for sustainable development and beekeeping productivity.



### OP-085

#### Considerations when sampling honey bee (*Apis mellifera*) colonies for volatile organic compounds (VOCs) associated with honey bee diseases, pests and environmental contamination

Maggie C Gill<sup>1</sup>, Bruce King<sup>2</sup>, Dennis vanEngelsdorp<sup>3</sup>, Falko Drijfhout<sup>1</sup>

<sup>1</sup>Lennard-Jones School of Chemical and Physical Sciences Lennard-Jones Building Keele University Staffordshire ST5 5BG, UK

<sup>2</sup>US Army DEVCOM Chemical Biological Centre (CBC), Aberdeen Proving Ground, MD, USA

<sup>3</sup>University of Maryland, College Park, MD, USA

It has long been established that odour and pheromones in the form of volatile and semi volatile organic compounds play an important role in honey bee colonies. Honey bee diseases such as American foulbrood (*Paenibacillus larvae*) and pests such as *Varroa destructor*, Small Hive Beetle (*Aethina tumida*) and *Tropilaelaps* spp. cause significant colony losses globally each year. Diseases and pests produce specific VOCs and cause behavioural changes within a colony, therefore altering the colony VOC profile. Due to their wide-ranging foraging activity honey bees collect VOCs from the local environment in the form of nectar, propolis, water and electrostatic particles. Hive samplers collect these VOCs indiscriminately, and therefore analysis yields valuable data related to both the colony and the local environment.

Honey bee colonies located at the University of Maryland, USA, were sampled using a variety of samplers (PSP, MonoTraps and Tenax TD) positioned at different location within colonies and for different time periods. GCxGC-TOFMS was used to determine the most effective sampler, position in hive and duration of sampling to detect VOCs associated with either diseases, pests or environmental contamination. Data analysis showed that the locations of samplers within colonies had no impact on the quantity or peak area of VOCs detected. When the data for VOCs associated with honey bee were analysed the number and peak area of VOCs detected increased with duration of sampling but decreased VOCs associated with environmental contamination were analysed. The peak area of honey bee and environmental VOCs detected varied with the type of sampler used. MonoTraps consistently detected the smallest peak areas, with PSPs and Tenax TD being comparable for honey bee VOCs and Tenax TD detecting the largest peak areas for environmental VOCs.

These results have demonstrated the type of sampler and location in hives that have the lowest burden on colonies and beekeeper to effectively detect specific VOCs. Further research will be undertaken to identify honeybee disease and pest biomarkers which could be used for early detection and treatment and to also ascertain the level and distribution of environmental contamination to improve decontamination.

### OP-086

#### Approach to the epidemiology of varroosis in Central Chile apiaries

María Mayda Verde Jiménez<sup>1</sup>, Marnix Alexander Doorn<sup>1</sup>, Leslie Vallejos<sup>1</sup>, Verónica Rachel Olate Olave<sup>2</sup>,

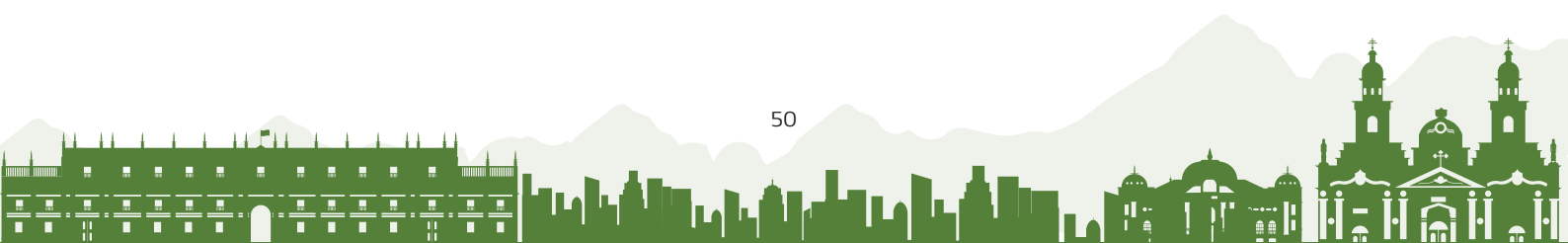
Jorge Demedio Lorenzo<sup>3</sup>

<sup>1</sup>Fundación UC Davis-Chile Life Sciences Innovation Center

<sup>2</sup>Instituto de Investigación Interdisciplinaria (I3), Universidad de Talca, Campus Lircay, Talca, Chile

<sup>3</sup>Facultad de Ciencias Veterinarias, Universidad Agraria de La Habana "Fructuoso Rodríguez", La Habana, Cuba

Varroosis is a parasitic disease affecting honeybees caused by *Varroa destructor* mites. Its epidemiology is associated with several factors. Its control depends not only on environmental or genetic aspects but also on management practices. To identify some epidemiological aspects associated with varroosis in colonies from the Central Region of Chile and to establish possible relationships between them, 58 apiaries located in the Region of Valparaíso, Metropolitana, and O'Higgins were studied, during years the 2015 and 2016. The apiaries were monitored five times in different seasons. Factors related to management practices by beekeepers were incorporated through a qualitative survey. Infestation rates by *Varroa* sp. and the honeybee colony strength were measured in each case. Results indicated that infestation rates by *Varroa* sp. are variable and correlated with some management practices, especially with the application of varroicidal treatments and with additional feeding (energy and protein food supplements). Despite beekeepers' efforts to treat their colonies, the global prevalence of varroosis was close to 53%. This indicates that to effectively control this parasitosis, additional and standardized methodologies must be included, taking a holistic point of view, including selection, genetic improvement, biotechniques to control the mites, and in specific cases, coordinated territorial treatments.





### OP-088

#### Proteomics screening for honey bee health

Renata Moravcova<sup>1</sup>, Kyung Mee Moon<sup>1</sup>, Greg R. Stacey<sup>1</sup>, Jason C. Rogalski<sup>2</sup>, Xiaojing Yuan<sup>2</sup>, Stephen F. Pernal<sup>3</sup>, Marta M. Guarna<sup>3</sup>, Shelley Hoover<sup>4</sup>, Ida M. Conflitti<sup>5</sup>, Amro Zayed<sup>5</sup>, Robert Currie<sup>6</sup>, Pierre Giovenazzo<sup>7</sup>, Mateus Pepinelli<sup>5</sup>, Leonard J. Foster<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada

<sup>2</sup>Proteomics Core Facility, University of British Columbia, Vancouver, Canada

<sup>3</sup>Agriculture and Agri-Food Canada, Beaverlodge, Canada

<sup>4</sup>Department of Biological Sciences, University of Lethbridge, Lethbridge, Canada

<sup>5</sup>Department of Biology, York University, Toronto, Canada

<sup>6</sup>Department of Entomology, University of Manitoba, Winnipeg, Canada

<sup>7</sup>Department of Biology, Université Laval Quebec, Quebec City, Canada

Although honey bees are crucial to world agriculture, their health has declined over recent decades due to different stressors. Unfortunately, identifying the stressors impacting bee health without post-mortem analysis within a colony is difficult. By measuring changes in the transcriptome, proteome, and microbiome induced by known stressors, we aim to identify diagnostic markers to help develop a health assessment tool powered by stressor-specific biomarkers (BeeCSI). This study focuses on the proteomics contingent of a project for Canadian honey bee health monitoring and management.

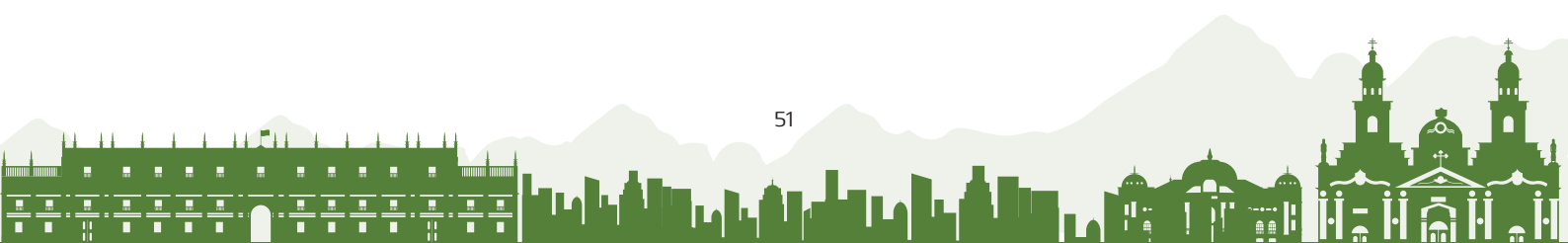
Laboratory and field studies were carried out in Canada across five provinces. Honey bees were naturally or experimentally exposed to different stressors, including parasites, pathogens, agrochemicals, nutrition restriction, and the eleven most common two-way combinations of stressors cross-compared for effects. Five biological replicates (colonies) per condition and eight crop systems (four of which were studied longitudinally over two years) were studied, totaling >2500 samples. Both control and affected bees were sampled with collected metadata. To obtain quantitative data, dissected, digested bee tissues (head, abdomen, gut) were analyzed by state-of-the-art mass spectrometry.

Data collected for the first subset (1377 of 2577 samples) includes treatments with five (of six) parasites and pathogens, five agrochemicals (themselves and within mixtures), two nutrition restriction stressors, and all eight crop systems. The data identified ~5800 protein groups with an average of ~4700 protein quantifications per sample, leading to the richest proteomics data set on honey bees to date.

The first analyses show no significant effects on protein abundance due to provinces of origin. However, including cage/colony and tissue specificity highlighted differences between tested relationships (protein amount~dose+colony+tissue).

When tested within specific tissues, we observed ~2300 ( $q < 0.05$ ) differentially expressed proteins across all experiments, where the maximum number of significant proteins within an experiment was 600. However, when testing without tissue specificity, only ~24 significant proteins were detected across all experiments, emphasizing the importance of tissue-specific analysis.

Upon completion, we will compare the effects of stressors based on collected metadata (e.g., province origin, type of experiment – colony/cage, treatment dosage, disease severity), identifying significant stressor-specific markers and conditions to develop a new bee health assessment tool.



### OP-089

#### Honey bee gut microbiota dynamics during starvation and recovery

Daniel Juarez Payes<sup>1</sup>, As. Prof. Charlene Kahler<sup>2</sup>, Dr Matthew Morgan<sup>3</sup>, Prof. Ian Small<sup>1</sup>, Dr Julia Grassl<sup>1</sup>

<sup>1</sup>School of Molecular Sciences, The University of Western Australia, Australia

<sup>2</sup>Marshall Centre, School of Medical Sciences, The University of Western Australia, Australia

<sup>3</sup>Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia

The decline of honey bees has become a global concern, and nutrition plays a critical role in honey bee health and productivity. When nutritional requirements are not met, malnutrition and starvation can affect the health of the entire colony and its long-term productivity. Even under optimal conditions, colonies affected by starvation are unable to recover their weight, indicating a disruption of the overall health of the colony. Recovery may be halted as a consequence of a disrupted microbiome in the honey bee guts or the whole colony. Beekeepers in the field mitigate the effects of starvation by replacing queens and/or capped brood frames from a healthy colony to a struggling one. Inadvertently, they may transfer a healthy mixture of gut/hive microbes that restore a healthy microbial community in the affected colony. In this study, we explore the effects of starvation on the honey bee gut microbiome and the potential for microbial recovery through a transfer of healthy capped brood frames. By utilizing deep sequencing of the 16S gene and shallow shotgun metagenomics, we monitor the bacterial gut community dynamics and metabolic functional changes in the gut environment. Our findings will contribute to the development of optimal supplementary feeding strategies for promoting honey bee health through better nutrition. Finding optimal strategies for promoting honey bee nutrition and gut health is essential for sustaining honey bee populations and global food production.

### OP-090

#### Effect of an electrical pulse on the control of *Varroa destructor* in *Apis mellifera*

Andrea Julieta Müller Sepúlveda<sup>1</sup>, Javiera Gacitúa Cortés<sup>2</sup>

<sup>1</sup>Instituto de Ciencias Agroalimentarias, Animales y Ambientales, Universidad de O'Higgins

<sup>2</sup>Escuela de Ciencias Agroalimentarias, Animales y Ambientales, Universidad de O'Higgins

Today, honey bees are the most important group of pollinators, being responsible for agrobiodiversity and 76% of the crops consumed by humans in the world. In Chile, the O'Higgins region is the region with the second largest number of hives in the country and is the largest honey exporting region in the country. Despite productivity, biotic and abiotic stressors cause significant colony losses, the most important of which are the spread of diseases and parasitism. The global picture places the *Varroa destructor* mite as the main threat to bee health, while acaricide treatments can cause sub-lethal effects in bees and intoxication in humans, as well as generating resistance to the use of these compounds. In order to control this mite without the use of acaricidal compounds, we proposed the evaluation of the effect of an electric current through a device placed at the entrance of the hives as a sustainable deworming method. Its efficacy in controlling mite health and bee health safety was determined. For this purpose, the treatment was evaluated in 12 experimental hives (9 treated and 3 as control group) for 13 weeks. Three treatment schedules with ranges between 1 - 5 mA were implemented, evaluated as continuous pulse, pulse every 60 seconds and pulse every 1 second. The variables evaluated were the count of fallen mites and bee mortality, the response of the bees and the propolis coating of the devices. The results show that the efficacy of the electric pulse as an anti-parasitic method could not be demonstrated and electricity is perceived to accelerate bee mortality under the variables studied. It is suggested to evaluate honey bee stress by other parameters, such as determination of the enzyme ornithine decarboxylase and heat shock protein, temperature and internal relative humidity of the hive, to empirically identify whether mortality is directly related to the electrical exposure of the pulse control device.



## Bee Health

### OP-091 [Bee Health]

#### Biometric parameters of newborn drones (*Apis mellifera* L.) and factors that affect them in hives of Mayabeque, Cuba

Leyanis Ocaña Nápoles, Anisley Perez Hernandez, Tamara Fernández Gómez, Jorge Demedio Lorenzo

Universidad Agraria de La Habana, Mayabeque, Cuba

The morphometric method became a much more exact science and is now capable of differentiating species and races by describing some fifty characters, which is why it is considered a rapid and effective primary diagnostic method, although in Cuba, as in many other countries, insufficient attention has been paid to the study of drones. The individuals in the hive are susceptible to diseases such as varroosis, caused by the Varroa destructor mite (Anderson & Trueman), capable of negatively influencing weight and dimensions, so that it is of maximum interest to evaluate their infestation rates. The objective of the work was to determine biometric parameters of *Apis mellifera* L. drones at birth and some factors that affect them, including parasitism by this mite. The research was carried out in three apiaries in the San José de las Lajas municipality and included a monthly sampling of 10 hives/apiary in November and December 2015 and January 2016. In each hive, a 10x10 fragment of honeycomb with drone brood was cut. cm and placed in a total visibility incubator at 34 °C and relative humidity of  $95 \pm 2$  %, to examine 50 individuals at birth / hive and their cells of origin. The diameter of 10 cells was averaged and the drones were determined with birth weight, body length, right forewing length and color. Extension and intensity of invasion by *V. destructor* were determined. The weight and dimensions of the drones coincided with those of the original European races of the current Cuban bee and were influenced by the apiary of origin, the month of sampling and the intensity of infestation.

### OP-092

#### Honeybee colony loss in Kenya

Malena Sibaja Leyton<sup>1</sup>, Michael Lattorff<sup>2</sup>, Nkoba Kiatoko<sup>3</sup>, Fabrice Requier<sup>4</sup>

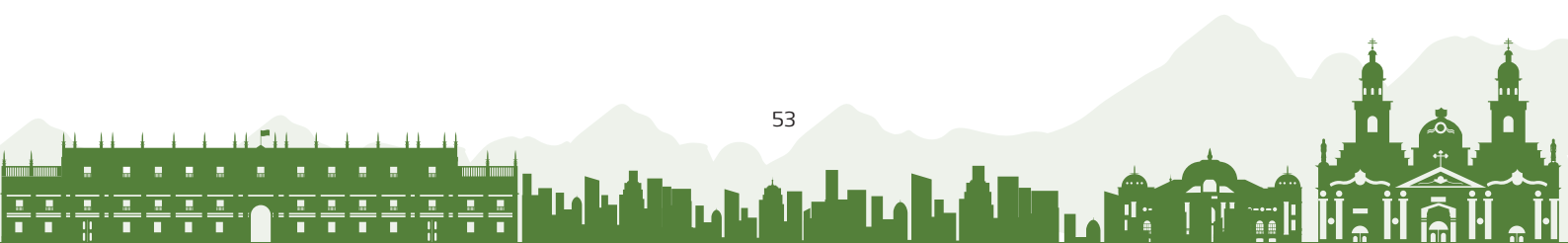
<sup>1</sup>Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France., International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya

<sup>2</sup>Department of Chemistry, University of Nairobi, Nairobi, Kenya.

<sup>3</sup>International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya.

<sup>4</sup>Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, Gif-sur-Yvette, France.

Global concerns about colony losses of honeybees, *Apis mellifera*, over the last decades encouraged large-scale programs to monitor colony losses in different regions, however, sub-Saharan Africa (SSA) is missing. Research from field studies and monitoring programs have defined groups of stress factors influencing colony losses, from which climate and beekeeping management are included. Using a standardized questionnaire, we established the first monitoring of the managed honeybee colony losses in Kenya. The monitoring concerned data from 2021-2022 and was divided into two semestrial surveys. A total of 595 responses were collected for the first, and 511 responses for the second semestrial survey, representing the largest data collection of honeybee colony losses in SSA. In this talk, we will present results of the two main objectives of our study, (1) the estimates of seasonal and annual colony losses of managed honeybees in Kenya, and (2) the exploration of potential influences of the interaction between climate and beekeeping management on these losses. We will discuss how our results can help local beekeepers reduce their colony losses and improve their livelihoods. Finally, this monitoring could encourage other countries in SSA to join and extend the program to complete the knowledge gap on colony losses for the region.



### OP-093

#### Validation of a method for estimating *Varroa destructor* parasitic level in *Apis mellifera* colonies

Elian Tourn<sup>1</sup>, Eliana Gabriela Revello<sup>2</sup>, Candela Lastiri<sup>2</sup>, Gabriel Gomez<sup>2</sup>, Luciano Marcelo Morales Pontet<sup>2</sup>, Giuliano Maximiliano Cernada<sup>2</sup>, Meredith Machado<sup>2</sup>, Joaquin Ignacio Cellichini<sup>2</sup>, Martin Villanueva<sup>2</sup>, Agostina Dambrosio<sup>2</sup>, María Sol Ballarati<sup>2</sup>

<sup>1</sup>Laboratorio de Estudios Apícolas (LabEA), Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina; Cooperativa de Trabajo Apícola Pampero Limitada, Bahía Blanca, Buenos Aires, Argentina.

<sup>2</sup>Cooperativa de Trabajo Apícola Pampero Limitada, Bahía Blanca, Buenos Aires, Argentina

*Varroa destructor* causes a parasitic disease that must be treated in order to maintain productive *Apis mellifera* colonies in most parts of the world. There are several methodologies designed to measure the level of its infestation in colonies. However, the "jar method" is one of the most commonly used techniques for rapidly and easily estimating the parasitic load per hive. In order to validate the method, the relationship between the real infestation (RI) and the estimated infestation (EI) was evaluated. For this purpose, 306 beehives were utilized and distributed among various apiaries located in different provinces throughout the Argentine Republic: Buenos Aires, Santa Fe, Córdoba, Mendoza and Tucumán. In each one, three brood frames were selected to collect samples of 300-400 adult bees. Afterwards, they were shaken with water and alcohol for several minutes, and the quantity of bees and mites in each sample was registered. Furthermore, to estimate the degree of development in each colony, the frames covered by adult bees (AB) and frames with brood (BB) were registered. Once this was done, technical floors and a sanitary treatment of recognized efficacy (Aluen CAP<sup>®</sup>, oxalic acid in slow-release strips) were used to obtain the actual parasite load. To collect mites, the technical floors were checked weekly for 42 days. As expected, a highly significant regression was obtained between the real and estimated infestation, with the formula:  $RI = 280.89 EI + 859.59$  ( $F_{1, 305} = 106.48$ ;  $R^2 = 0.25$ ;  $p < 0.0001$ ). However, a more fitted regression model was found by including the colony development variables, resulting in:  $RI = -9.23 EI_2 + 484.81 EI + 12.73 AB_2 - 316.65 AB - 64 BB_2 + 754.14 BB + 420.11$  ( $F_{6, 305} = 26.44$ ;  $R^2 = 0.35$ ;  $p < 0.0001$ ). The results suggest that the "Jar method" is capable of providing a reliable estimate of the mite load in a colony. Furthermore, including colony development variables in the estimation process can improve the accuracy of the results. However, for scientific efficacy trials, it is imperative to use the total number of mites collected in colonies with technical floors to ensure reliable calculations.

### OP-094

#### Peek a Boo: Manipulation and Field-sampling of the Small Hive Beetle

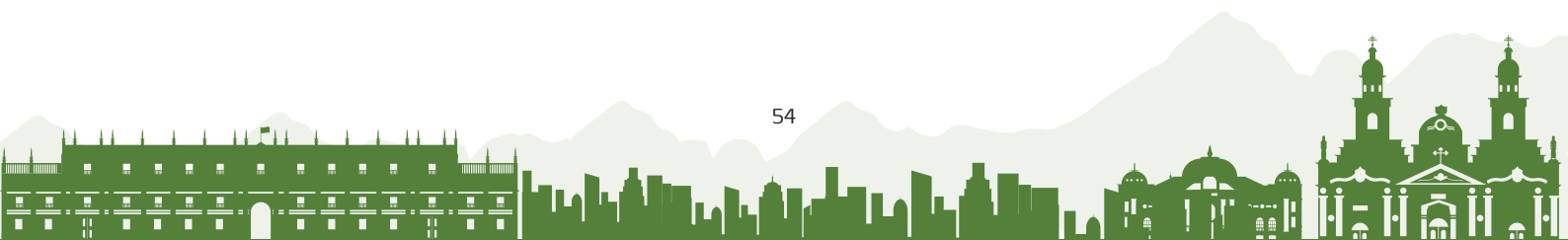
Ethel M Villalobos<sup>1</sup>, Luis A Medina Medina<sup>2</sup>, Zhening Zhang<sup>1</sup>, Scott Nikaido<sup>1</sup>, Emanuel A Miranda<sup>3</sup>, Jason Wong<sup>1</sup>, Jessika Santamaria<sup>1</sup>

<sup>1</sup>Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI, USA

<sup>2</sup>Departamento de Apicultura, Universidad Autónoma de Yucatán, Campus de Ciencias Biológicas Y Agropecuarias, Mérida, Yucatán, Mexico

<sup>3</sup>Centro de Investigaciones Apícolas Tropicales, Programa Integrado de Patología Apícola, Universidad Nacional, Heredia, Costa Rica

In this study we compared the relative infestation and in-hive distribution of the small hive beetle, SHB, (*Aethina tumida*) for 3 sites: Hawaii, Mexico, and Costa Rica. We found that SHB relative infestation can be quite variable within and across apiaries, however, infestation levels did not seem to be influenced by whether the bees were Africanized or European. Daylight, smoke, and physical manipulation of the frames during inspection encourages the SHB's evasive behaviors. SHB capture rates on the floor of "inspection disturbed" hives were significantly higher than in colonies at "rest", suggesting that the majority of SHB do not naturally congregate on a bare bottom board but move downward in response to colony disturbances. In this study, the capture of adult beetles at the bottom board before and after inspection was done by hand, and it did not include traps or refugia on the floor of the hive. It's possible that providing hiding areas on the floor of the hive may affect the in-hive distribution and capture rates of SHB in "at rest" colonies. Our results also showed that the sum of SHB adults captured on the top areas of the hive (cover and side frames) was significantly correlated with the overall number of beetles captured/colony. Hive configuration influenced SHB distribution, and taller hives (with 2 honey supers) had lower capture rates of SHB on the top areas of the hive, which were not correlated with overall SHB density/hive. This result suggests that our inspection protocol could be used as an alternative method to assess the relative SHB infestation in small to medium sized colonies. This targeted screening provided immediate information about the relative SHB infestation, and the beetle survey can be incorporated to regular hive inspection, it does not involve follow-up visits, and requires less time and materials than other methods. Consequently, this technique may be more user friendly and allow beekeepers to conduct inspections more frequently and respond more quickly to changes in SHB density.



### OP-095

#### Recommendations for oxalic acid application method and treatment intervals for reduction of *Varroa destructor* populations in western honey bee (*Apis mellifera*) colonies

Cody Prouty<sup>1</sup>, Hossam F. Abou Shaara<sup>2</sup>, Branden Stanford<sup>3</sup>, James D. Ellis<sup>1</sup>, Cameron J. Jack<sup>1</sup>

<sup>1</sup>Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

<sup>2</sup>Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, Egypt

<sup>3</sup>Bureau of Plant and Apiary Inspection, Florida Department of Agriculture and Consumer Services, Gainesville, FL, USA

Oxalic acid (OA) is a popular miticide used to control *Varroa destructor* in western honey bee (*Apis mellifera*) colonies. Several methods of OA application and different treatment intervals are recommended by practitioners and researchers. Unfortunately, these applications have varying efficacies. Our research aim was to investigate which method of OA application (dribbling, fogging, or vaporizing) was the most effective at reducing *V. destructor* infestations (Experiment 1) and to improve upon this method by determining the treatment that resulted in the greatest *V. destructor* control (Experiment 2). In both experiments, we used the product Api-Bioxal (97% OA) and maintained 40 honey bee colonies (10 per treatment) in single deep, 10-frame Langstroth hives for all treatments. In Experiment 1, our three methods of application included: 1) dribbling 50 ml of 3% OA solution, 2) vaporizing 4 g solid OA, and 3) using an insect fogger supplied with 2.5% OA dissolved in ethyl alcohol. Each treatment was applied three times at 7-day intervals. A fourth control group that received no treatment was also included in the study. After three weeks of OA applications, only the vaporization method reduced *V. destructor* infestations (from 9.24 mites/100 bees pretreatment to 3.25 mites/100 bees post treatment) and significantly increased the amount of brood and number of adult bees. In Experiment 2, all colonies were treated with four applications of OA via vaporization at a constant concentration of 4 g OA/colony. In this experiment, the groups were separated by treatment intervals at either 3-day, 5-day or 7-day intervals. Another ten colonies were designated as the negative controls and received no treatment for *V. destructor*. We observed that both 5-day and 7-day intervals significantly reduced *V. destructor* populations from pretreatment levels over that of the controls, while 3-day intervals did not. Additionally, OA application at 7-day intervals was the only treatment group that dropped significantly more mites in subsequent applications. Our data supports that OA can be used to reduce *V. destructor* infestation and that vaporizing OA at 4 g every 5 - 7 days is the most effective method of application.

### OP-096

#### Prevalence and co-occurrence of honey bee-associated pathogens in native bees and wasps

Kaitlin Deutsch<sup>1</sup>, Jason Graham<sup>2</sup>, Humberto Boncristiani<sup>3</sup>, Tomas Bustamante<sup>4</sup>, Ashley Mortensen<sup>5</sup>, Daniel Schmehl<sup>6</sup>, Ashlyn Wedde<sup>7</sup>, Dawn Boncristiani<sup>8</sup>, Jay Evans<sup>8</sup>, James Ellis<sup>9</sup>

<sup>1</sup>Department of Entomology, Cornell University, Ithaca, New York, USA

<sup>2</sup>Planet Bee Foundation, San Francisco, California, USA 94132

<sup>3</sup>Inside The Hive Media, Consulting Inc., Odenton, Maryland, USA, 21113

<sup>4</sup>Independent collaborator, Dallas, Texas, USA

<sup>5</sup>The New Zealand Institute for Plant and Food Research Limited, Hamilton, New Zealand

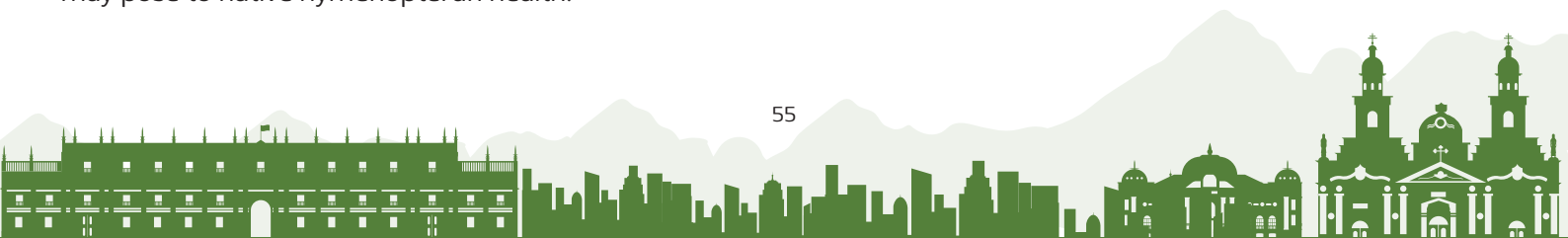
<sup>6</sup>Bayer CropScience LP, 700 Chesterfield Pwky. W., Chesterfield, Missouri, USA

<sup>7</sup>Driscoll's Global R&D, Watsonville, California, USA

<sup>8</sup>Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, USA

<sup>9</sup>Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

Insect pollinators have experienced significant declines and range contractions in the past decade. While the drivers of these declines are complex and interacting, there is growing evidence that infectious diseases are a key factor in the deteriorating health of pollinators. Much of our knowledge on the effects of pathogens on pollinator health comes from studies on managed Western honey bees, *Apis mellifera*. However, recent research has demonstrated that pathogens associated with honey bees are shared by other pollinators and can negatively affect their health as well. We surveyed honey bees and 15 native bee and wasp species for 13 pathogens traditionally associated with honey bees. Eleven of the species sampled, including several commercially important native pollinators, have not been screened before for honey bee-associated pathogens. We found at least one honey bee-associated pathogen in 53% of native bee and wasp samples. The most widely distributed and commonly detected pathogens were *Vairimorpha* (formerly *Nosema*) *ceranae* (detected in 18% of native bees and wasps), *Melissococcus plutonius* (24%), *Ascospaera apis* (9%), Deformed wing virus (8%) and Black queen cell virus (9%). The prevalence of viruses was generally higher in honey bees than in native bees and wasps. However, the prevalence of *M. plutonius* and *A. apis* was significantly higher in some native bee species than in honey bees, indicating that many non-viral pathogens are widely distributed and more commonly found in native bees and wasps than previously thought. Often, multiple pathogens occurred in a single individual for many bee/wasp species (including honey bees), with some species having up to four pathogens co-occurring in a single individual. The patterns of co-occurrence suggest potential mutualisms and/or antagonisms at the pathogen community level. Our data do not tell us about the impact the identified pathogens may have on native bee and wasp health. Yet, trends in pathogen prevalence are the first step toward evaluating the risk posed by pathogen spillover between managed and native pollinators. We recommend that the commonly detected pathogens in this study be included in subsequent pathogen surveys and in experimental inoculations to determine the risk they may pose to native hymenopteran health.





### OP-097

## VarroaAPPacus - A Novel Smartphone- and Machine Learning-Based Approach for Automated Detection and Counting of Dead Varroa Mites for Pest Control

Joost Janssen, Oliver Theel

Department of Computer Science, Carl von Ossietzky University, Oldenburg, Germany

Varroa mites present one of the greatest threats to the western honey bee and in order to successfully control them, the population of Varroa mites in a bee hive must be monitored. Monitoring is often done by inspecting and by counting the dead Varroa mites that have fallen onto an inspection board right underneath the bee hive. This counting and thereby the identification of the number of dead mites is a time-consuming but beneficent task: depending on this count, subsequent pest control measures can be initiated and thereby the health of the bee population be heavily increased. Generally speaking, the more often this counting is performed, the better is Varroa mite control. But unfortunately, the counting process is quite time-consuming and error-prone. Furthermore, it does not scale well with the number of bee hives managed by a single bee keeper. Thus, an automated counting process that is fast, easy to adopt in the field, and as reliable as possible, is needed.

We propose an automation based on a machine learning algorithm, that performs object detection. The resulting automated Varroa mite counting service is then provided to the bee keeper via a smartphone app. We have identified a suitable algorithm based on partially pre-trained models that are related to the detection of small objects (like Varroa mites). For the training of the model, a novel training dataset has been created. It consists of more than 800 photographs with sometimes thousands of -now labeled- dead Varroa mites lying on inspection boards. The developed smartphone application allows to take photos of inspection boards or processes photos taken by other means and automatically counts as well as graphically labels the detected Varroa mites. Photographs and counting results are furthermore persistently managed by the smartphone application on a per-bee hive basis, allowing for easy use by the bee keeper.

The described system has been implemented and is running. Although it is in prototype state with many opportunities for improvement in functionality and preciseness, it is already fully functional and powerful enough to efficiently and effectively assist the bee keeper in Varroa mite control.

### OP-098

## Transportation sensitizes honey bees to insecticides

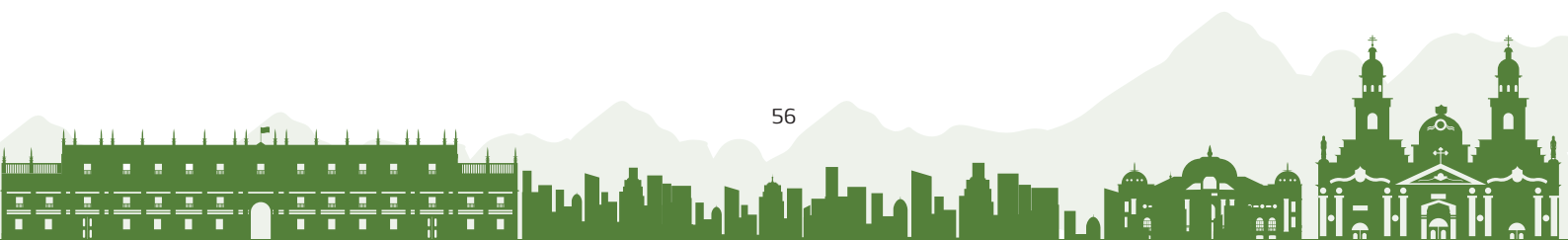
Zachary Huang<sup>1</sup>, Shudong Luo<sup>2</sup>

<sup>1</sup>Huang, Z.Y.

<sup>2</sup>Luo, S.

Honey bees are critical pollinators whose managed populations face global declines due, in part, to the widely use of insecticides. Transportation of honey bees is necessary all over the world because of the great demands of fruit and vegetable pollination. Previous studies have shown that transportation causes negative impact on honey bee physiology by causing smaller food glands and increased incidences of a fungal pathogen *Nosema ceranae*. More and more studies suggested that the losses were driven by multiple facts such as insecticides interacting with parasites and pathogens. However it was not known the interactive effects of transportation and insecticides.

We show that transportation and simulated transportation both render honey bees more sensitive to neonicotinoid insecticides. We found that transportation makes bees more sensitive. We hypothesized that transportation made bees aging faster and the bees will become more sensitive to insecticides with age. This hypothesis was confirmed in the next experiment because foragers were the most sensitive bees among newly emerged bees, nurses and foragers. In a third experiment, we discovered that simulated transportation increased juvenile hormone levels significantly compared to un-transported bees. These results suggest that transportation changed hormonal titers of bees, making them more like foragers, thus increasing their sensitivity to insecticides. Our study suggests that the widely practiced transportation of honey bee colonies has severe negative impacts on honey bee physiology, especially for making them more sensitive to insecticides.



### OP-099

#### Using a honey bee cell line as an approach for studying the effects of fungicides

Michael Goblirsch<sup>1</sup>, Dalma Martinović Weigelt<sup>2</sup>

<sup>1</sup>United States Department of Agriculture, Agricultural Research Service, Poplarville, MS, USA

<sup>2</sup>University of St. Thomas, Biology Department, St. Paul, MN, USA

Fungicides are a necessary tool for the control of plant fungal pathogens; however, their use on flowering crops can be at odds with honey bees that visit these crops for food resources. Although once considered innocuous for honey bees, there is increasing evidence that chronic, sublethal exposure to fungicides can result in negative effects on honey bee development, behavior, and responses to infectious diseases. Our understanding of how fungicides detract from honey bee health is often approached through field and caged-bee studies. While these approaches work well for investigating organismal and social responses to fungicides, less intrusive methods that provide a high level of control and resolution are needed to explore mechanisms of cellular toxicity and host-pathogen interactions in response to field-relevant exposures. Cell lines provide a convenient, highly controllable platform for studying the impact of fungicides on cell health and can complement whole bee and colony-based findings. Cell lines are routinely used in human and animal toxicology but are surprisingly lacking for honey bee research. This deficiency prompted us to use a continuous cell line derived from honey bee embryonic tissues, AmE-711, to characterize the cellular response of select fungicides commonly applied to flowering crops that honey bees may visit. We demonstrate that exposure of AmE-711 honey bee cells to a range of concentrations of commonly used fungicides (i.e., pyraclostrobin and chlorothalonil) negatively affected honey bee cell viability. Moreover, exposure of AmE-711 cells to pyraclostrobin inhibited cellular respiration via alteration of the electron transport chain. These cellular level effects are concerning, as they could manifest as metabolic and thermoregulatory dysfunction at the individual bee and colony levels. Use of AmE-711 in toxicological research can aid assessment of the risks that fungicides, as well as other pesticides, pose for honey bee health, and could promote development of chemistries with reduced environmental impacts.

### OP-100

#### Management practice against small hive beetle as a source of microplastic contamination in honey bee colonies

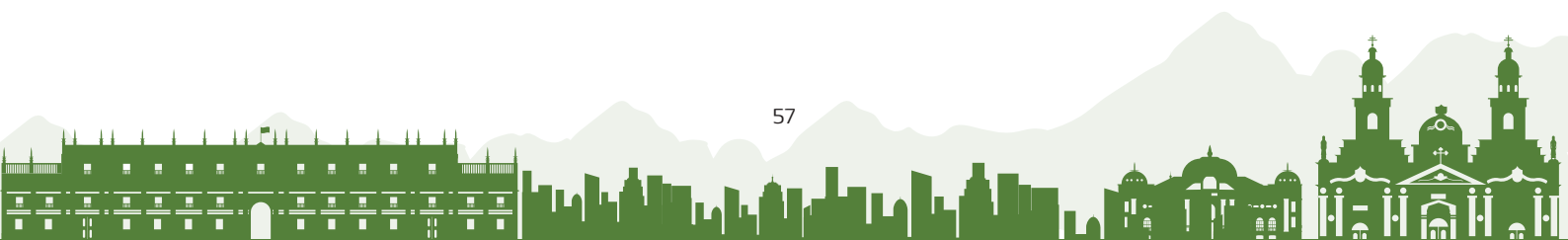
Micaela Buteler<sup>1</sup>, Marina Alma<sup>1</sup>, Juan Pablo Tomba<sup>2</sup>, Ethel M Villalobos<sup>3</sup>

<sup>1</sup>Instituto de Investigaciones en Medio Ambiente y Biodiversidad (INIBIOMA)-CONICET-Universidad Nacional del Comahue, Bariloche, Argentina

<sup>2</sup>Instituto de Investigaciones en Ciencia y Tecnología de Materiales CONICET, Mar del Plata, Argentina

<sup>3</sup>Plant and Environmental Protection Sciences, (CTAHR). University of Hawaii at Manoa, Honolulu, Hawaii, USA

Microplastics (MP) have emerged as a widespread environmental contaminant affecting bee health. In this study we report on the impact of one of the cultural practices used to control the small hive beetle (*Aethina tumida*). Management of the beetle often includes the use of in-hive traps of different kinds, one technique involves the introduction in the hive of non-woven microfiber wipes. When placed inside the hive, bees chew on these wipes as they attempt to remove the foreign object. The bees' manipulation makes the wipes become fuzzy and fray to the point where beetles become entangled in their fibers. The current study aimed to examine the composition of these microfiber sheets and to evaluate whether their use resulted in unintended MP contamination of bees and honey. The experiment consisted of treating hives with one blue microfiber sheet placed on top of the frames for at least three months. After that time, we collected adult bees and honey samples from treated hives, control hives in the same apiary, and control hives in an apiary 7.5km away. Hives treated with the microfiber sheets had a significantly greater number of blue microfibers in the gut and cuticle of bees, than the control hives located in a different apiary. However, the control and treated bees located in the same apiary had a similar number of blue microfibers, which had a significantly greater number of fibers than the control bees that were in a different apiary. Honey from treated hives had a significantly greater number of blue MF than honey from the control hives. We showed that these fibers are an entanglement of polyethylene microfibers found in the bees cuticle, gut, and honey. Thus, the current study raises concerns of the use of microfibers sheets to trap the SBH as it results in the incorporation of microfibers into the ecosystem and into the food chain.



### OP-101

#### The impact of the varroacide VarroxCare® on the cardiac activity of Varroa destructor mites during laboratory experiments and efficacy assessment, impact on colony development and honeybees under field trials in Greece

Konstantinos Ilanidis<sup>1</sup>, Paulo Mielgo<sup>1</sup>, Max Watkins<sup>1</sup>, Alexandros Papachristoforou<sup>2</sup>

<sup>1</sup>Vita Bee Health, Basingstoke, UK

<sup>2</sup>Department of Animal Science, University of Thessaly, Larissa, Greece

VarroxCare® is an acaricide formulation, manufactured from oxalic acid and glycerin, through a unique heat process of the mixture and careful and controlled impregnation system. It presents prolonged action, and it has been reported as highly effective against Varroa destructor. Fiber-board strips impregnated in the mixture, result in a veterinary product which is introduced in hives. The present study assesses the efficacy of VarroxCare® under laboratory and field conditions in Greece. During laboratory assessment, a novel bio-assay which can evaluate the impact of any tested product on the cardiac activity of Varroa mites, was conducted. VarroxCare®, induced acute malfunction of the Varroa's cardiac activity, in terms of frequency and duration of the cardiac pulses, which led to the death of all examined mites within an average period of 11.12 minutes.

Field trials were performed in autumn of 2021 and winter of 2022, in Greece. In 2021, VarroxCare® was applied in a batch 10 colonies, naturally infested by Varroa destructor. Dead mites were counted daily with the use of a screen bottom board and possible effect on bee mortality was evaluated with the use of Gary traps. Furthermore, the strength of colonies (population and brood area) was also assessed. After a 56-day period of application, remaining mites were evaluated using Apistan and Apivar and the efficacy of VarroxCare® was assessed and compared with a batch of control colonies (8 untreated colonies). The efficacy was 93.60%, differed significantly from the natural mite fall of control colonies (11.66%). In 2022 VarroxCare® were applied a batch of 8 colonies and efficacy was assessed according to 2021 protocol. Efficacy was compared to the registered green acaricide, Varromed, applied also in 8 colonies. The strength of colonies and tolerance on honeybees was also evaluated like in 2021. Results showed a high efficacy of VarroxCare® against Varroa, reaching 97.12%, presenting no significant differences compared to Varromed (94.63%). No differences in colony strength and honeybee mortality were observed between experimental and control colonies. Overall, VarroxCare® appears as a promising, new "green" product for the control of *Varroa destructor*.

### OP-102

#### Apis mellifera colonies exposure to imidacloprid impairs larval cell structures

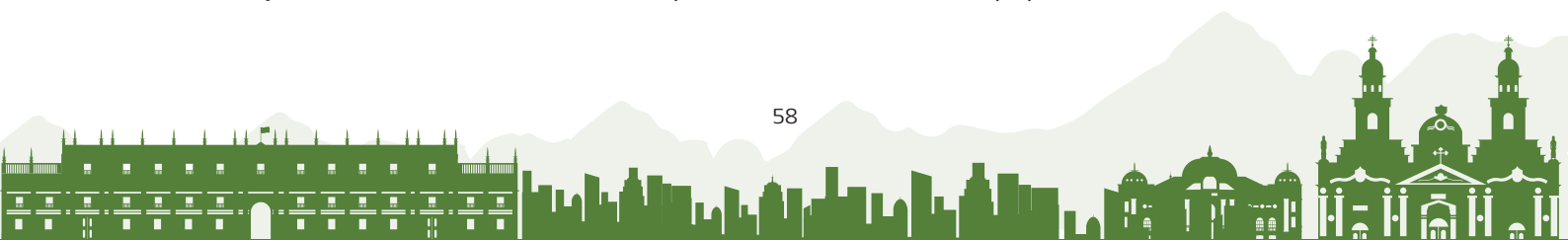
Daiani Rodrigues Moreira<sup>1</sup>, Tuan Henrique Smielewski De Souza<sup>2</sup>, Douglas Galhardo<sup>2</sup>, Cinthia Leão Figueira<sup>2</sup>, Breno Gabriel Da Silva<sup>3</sup>, Francieli Das Chagas<sup>1</sup>, Jean Samel Rocha<sup>2</sup>, Alessandra Fernandes Gonçalves Benites<sup>2</sup>, Vagner De Alencar Arnaut De Toledo<sup>2</sup>, Adriana Aparecida Sinópolis Gigliolli<sup>1</sup>, Maria Cláudia Colla Ruvolo-Takasusuki<sup>1</sup>

<sup>1</sup>Department of Biotechnology, Genetics and Cell Biology, Universidade Estadual de Maringá, Maringá, Brazil

<sup>2</sup>Department of Animal Science, Universidade Estadual de Maringá, Maringá, Brazil

<sup>3</sup>Department of Exact Sciences, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brazil

Pollination by bees is essential for the survival of native and agricultural plants. However, insecticides such as neonicotinoids can promote alterations in the organism of these insects. Thus, this study aimed to analyze the action of the neonicotinoid imidacloprid (IMD) in larvae of Africanized *Apis mellifera* honeybees after forty-two days of oral exposure in the field. For this purpose, we used three experimental groups in the apiary: control, commercial product imidacloprid (commercial IMD Evidence 700 WG®) and active ingredient imidacloprid (IMD active ingredient pestanal®) with six repetitions per group. Every three days, 300 mL of syrup composed of the insecticides at a concentration of 1 µg/L was provided. The control group received only syrup. After exposure of the colonies, the larvae were collected (n = 15, 3 to 5 days/larva), fixed, and prepared for histological and scanning electron microscopy evaluation. Exposure to the commercial insecticide IMD and the active principle IMD caused external changes in the larvae such as the absence of cuticular constrictions, undefined abdominal segments, and closed spiracles. In the treatment with IMD active ingredient, it was observed the tegument wrinkled and internally there was the detachment of the epithelium in relation to the musculature, formation of intercellular spaces with epithelial disorganization, detachment of the digestive cells showing granular and vacuolized cytoplasm, as well as the absence of regenerative cells. In exposure to the commercial IMD product, the changes were more pronounced with the larval midgut showing an increase in intercellular spaces and rupture of the epithelium with the release of the luminal contents, digestive cells showing granular cytoplasm with cytoplasmic protrusions protruding into the lumen and absence of regenerative cells. In both treatments the fat body exhibited more vacuolized trophoblasts and reduced in quantity, evidencing increased intercellular spaces and cell volume. Thus, contact with imidacloprid may impair the conditions of larval survival, since it compromised the whole internal structure of the insect, which consequently affects the colony since if the larvae do not develop there is no formation of pupae or adult insects.



### OP-103

#### Interaction of bacteria with gelsemine, nectar alkaloid toxic to honey bees

Vera Strogolova, Vyacheslav Strogolov

Strong Microbials Inc., Milwaukee, Wisconsin, USA

Honey bees rely on gut bacteria to detoxify some toxins found in nectar and pollen. One group of bacteria present in honey bee gut, honey and even propolis, is *Bacillus*. Work of USDA researcher Martha Gilliam identified 110 *Bacillus* species including *Bacillus subtilis* and *Bacillus pumilus* in honey bee gut. *Gelsemium sempervirens* or Caroline yellow jasmine is a vine native to the southern United States, Mexico, and Guatemala. It is the state flower of South Carolina. It usually blooms in early spring, but can start as early as December and bloom again in early fall. It is poisonous to people, livestock, and deer. Exposing honey bee queens to naturally occurring concentrations of gelsemine were reported to reduce egg-laying, but had no effects on adult bee mortality or resource consumption. We used Queen Monitoring Cages (QMCs) to explore the effects of *Bacillus* bacteria on gelsemine toxicity to honey bees. We hypothesize that oral administration of probiotic *Bacillus* bacteria to honey bees may ameliorate gelsemine toxicity and prolong lifespan of adult honey bees fed artificial diet of sugar and supplemental protein. This work explores the previously established role of microbiome in detoxification of dietary compounds.

### OP-104

#### Potential risk factors for honey bee health in Europe

Marc Oliver Schäfer<sup>1</sup>, Severine Matthijs<sup>2</sup>

<sup>1</sup>German NRL for Bee Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald - Insel Riems, Germany

<sup>2</sup>Belgian NRL for Bee Diseases, Sciensano, Brussels, Belgium

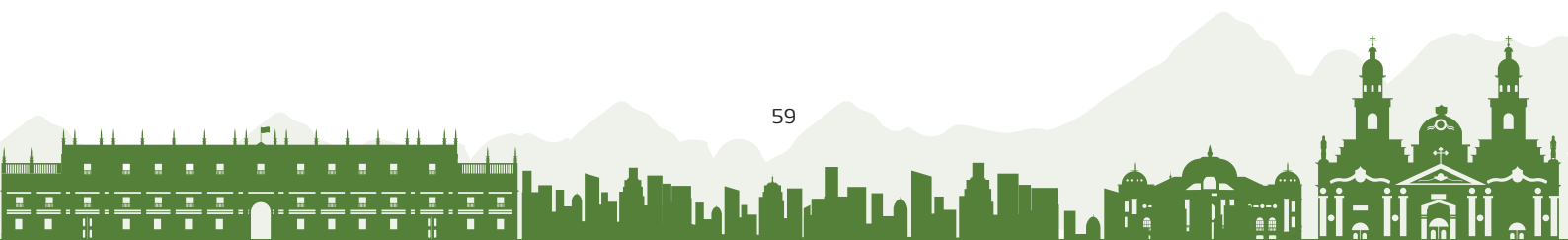
During the B-GOOD project (Giving Beekeeping Guidance by cOmputatiOnal-assisted Decision making), samples of honey bee colonies were analyzed for the presence of important pathogens and parasites. Together with various indicators - a flow of data from the colonies (e.g. weight, temperature, sound) and their environment (including landscape composition, resource availability, agricultural practices, climate) - a categorization of the health of a bee colony, the Health Status Index, will be accomplished. Here, we describe differences in the levels of detected parasites and pathogens during the project between the countries and the seasons.

Altogether, honey bee colonies from 15 European countries were sampled. Samples were taken three times per year: first in spring, when the bees start to forage; second in summer, when the colonies have reached their maximum size; and third in autumn before the over-wintering.

At eight apiaries in eight countries (BE, CH, DE, FR, GB, NL, PT and RO), eight colonies were sampled in 2020, 2021 and 2022. In five countries (CH, DE, FI, IT and NL) three colonies from eight beekeepers each were sampled in 2021 and 2022. In 2022, samples from three colonies each of 56 beekeepers from 11 countries (CH, BE, DE, FR, GR, IT, LV, NL, PL, PT and SE) were analysed.

For all samples the level of infestation with the parasitic mite *Varroa destructor* was determined and all were analysed for detection of BQCV, DWV-A, DWV-B, SBV and *Malpighamoeba mellificae*. Additionally, all spring samples were analysed for *Nosema apis*, *Nosema ceranae*, ABPV and CBPV; all summer samples were analysed for *N. apis* and *N. ceranae*; and all autumn samples were analysed for ABPV, CBPV, *Paenibacillus* larvae and *Melissococcus plutonius*.

In general, we detected only very few cases of foulbrood, high viral loads in almost all samples and as expected, rising numbers of *V. destructor* mites from spring to autumn. *N. apis* seems nearly vanished in Europe, while *N. ceranae* was frequently detected.



### OP-105

#### Trans-generational immune priming against American Foulbrood does not affect honeybee colony performance

Matti Leponiemi<sup>1</sup>, Miguel Moreno Torres<sup>2</sup>, Mikko Tiusanen<sup>3</sup>, Antoine Becker-Scarpitta<sup>4</sup>, Helena Wirta<sup>4</sup>, Dalial Freitak<sup>1</sup>

<sup>1</sup>Institute of Biology, University of Graz, Graz, Austria

<sup>2</sup>Institute of System Sciences, Innovation and Sustainability Research, University of Graz, Graz, Austria

<sup>3</sup>Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zürich, Switzerland

<sup>4</sup>Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland

As diseases are one of the major reasons behind the decline of honeybee health, studying the responses of honeybees against pathogens could provide ways to improve honeybee health. Immune priming, a function of the invertebrate immune system, can improve resistance against a previously encountered pathogen, and the priming effect can even extend to the next generation. The trans-generational immune priming (TGIP) has been shown to increase resistance of honeybee larvae against American Foulbrood (AFB) and has been proposed as a way to protect colonies from AFB outbreaks. Yet, what has remained unstudied is whether priming of the colony has any costs for the hives. It is widely accepted that immune function is often a trade-off against other life-history traits, hence immune priming could affect the colony performance.

In this experiment we set up 48 hives, half of them with queens primed by oral exposure to killed AFB bacteria and half as control. Through a two-year study we monitored the hives and measured their health and performance. We measured a number of hive health and performance parameters such as hive weight, brood amounts, honey yield, the prevalence of other common honeybee pathogens, and expression of relevant genes. We also studied the effects on foraging behavior by identifying the floral origins of honey and pollen samples by DNA-metabarcoding.

No effect of trans-generational immune priming was found on any of the hive parameters, such as queen failures, amount of brood, honey yield, pathogen prevalence, gene expression, or foraging choices. We did find other factors contributing to various hive performance parameters however. Time of the season, and also the apiary location, although only ten kilometers apart from each other, had an effect on the performance and health of the colonies, indicating that the local environment plays an important role in hive performance. We also found differences in the foraging of nectar and pollen, pollen being foraged from fewer but more variable sources, suggesting higher selectivity for pollen sources. Our results suggest that TGIP could serve as a safe tool in fighting the AFB in apiaries.

### OP-106

#### Nutritional supplements improves the strength and productivity of *Apis mellifera* colonies located in a *Eucalyptus grandis* plantation

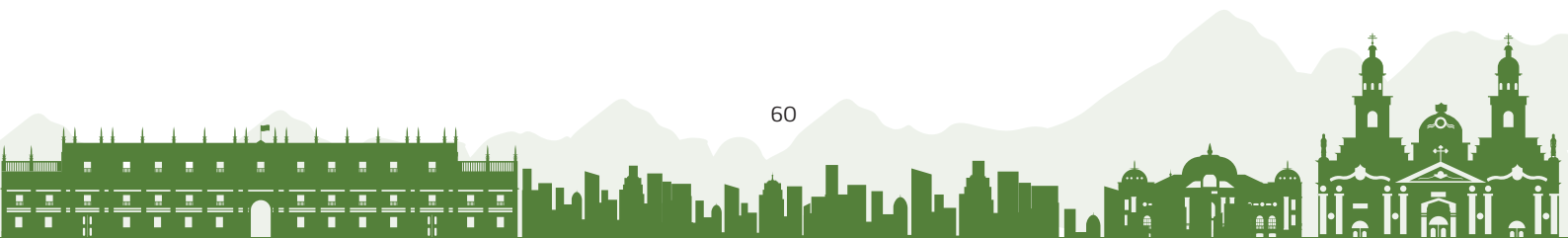
Matilde Alarcón<sup>1</sup>, Loreley Castellí<sup>1</sup>, Belén Branchiccela<sup>2</sup>, Ciro Invernizzi<sup>3</sup>, Karina Antúnez<sup>1</sup>

<sup>1</sup>Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay.

<sup>2</sup>Sección Apicultura, Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay.

<sup>3</sup>Sección Etología, Facultad de Ciencias, Montevideo, Uruguay.

Over the last years, there has been a significant increase in *Apis mellifera* colony losses worldwide, associated with nutritional problems, intoxication with pesticides or infection with pest and pathogens. In previous studies we demonstrated that nutritional stress and *Nosema* spp. infection has a severe impact on the colony strength. In this study we focused on the use of nutritional supplements to mitigate this problems. Five groups of 20 colonies were set in Colonia in summer (February), and received different treatments: i) control (no treatment), ii) polyfloral pollen patty (positive control of an adequate nutrition), iii) Apiprot (commercial product), iv) FeedBee (commercial product) and v) a homemade preparation based on beer yeast. In autumn (March), colonies were relocated into a *Eucalyptus grandis* plantation in Rivera, at the beginning of its flowering period. It is a natural scenario to study the nutritional stress, as *E. grandis* pollen have low crude protein percentage, low lipid content and deficiency in some aminoacids. All colonies had access to the pollen available in this plantation, but also received the designated supplements. At the end of the flowering period (May) colonies returned to Colonia. Colonies were inspected at the beginning (February), during and at the end of the flowering period (end of March and, May), and also in winter, to analyze the long-term effect (end of July). We analyzed the effect of the treatments on the colony strength (adult bee and brood population), its nutritional status, honey production and the infection level of *Nosema* spp. and *Varroa destructor* mites. The administration of nutritional supplements improved colony strength and individual nutrition status, when compared to the negative control group. In addition, homemade preparation, polyfloral pollen patty and Apiprot increased honey production. No effects of colony supplementation was observed on *Nosema* spp. or *V. destructor*. These findings suggest that timely nutritional supplementation of colonies can help to mitigate the severe damages causes by nutritional stress.





### OP-107

#### Bee pollen from native ulmo tree (*Eucryphia cordifolia*) improve of survival and reduce deformed wing virus variant A (DWV-A) load on honey bees

Diego Eduardo Silva Vasquez<sup>1</sup>, Marisol Vargas<sup>1</sup>, Nolberto Arismendi<sup>2</sup>, Richard Garcia<sup>1</sup>, Nelson Zapata<sup>1</sup>

<sup>1</sup>Universidad de Concepción, Facultad de Agronomía, Concepción, Ñuble, Chile

<sup>2</sup>Centro de Investigación Austral Biotech, Facultad de Ciencias, Universidad Santo Tomás, Valdivia, Chile

Deformed wing virus (DWV) in association with the *Varroa destructor* mite is one of the most important health problems in bee colonies in the world. There are no direct control strategies for the virus, the main strategy has been the varroa mite control. It is well known that a good nutrition is important mechanism for improve the defense against diseases in bees. In this sense, the bee pollen have been shown to have antimicrobial and antiviral activity; activity that have been related in most of case, to floral sources. Therefore, the aim of this study was to determine the effect of pollen ingestion in bees infected with DWV variant A. For this purpose, different types of pollen were collected from bee hives, being identified as: multifloral of fruit trees (1), multifloral of meadow (2), monofloral of mustard (3) and monofloral of native ulmo tree (4). For the assay, newly emerged bees were individually infected with a suspension of DWV-A ( $1 \times 10^9$  number of genomic copies per bee), locked in cages (n=50) and arranged in a bioclimatic chamber under controlled conditions (30 °C temperature, 60-70% relative humidity). After 48 h, 7 g of pollen was administered ad libitum, according to each treatment. Groups of inoculated and uninoculated bees were given a protein supplement and were used as controls. Four replicates per treatment were established. The survival of each treatment was evaluated daily for 20 days. Furthermore, every five days after virus inoculation, five bees were collected for quantification of viral load by qPCR. All pollen evaluated reduced the viral load (49% pollen 1, 40% pollen 2, 53% pollen 3 and 58% pollen 4) and increased the survival of virus-infected bees (65% pollen 1, 58% pollen 2, 60% pollen 3 and 70% pollen 4), when were compared to virus-inoculated bees fed with the protein supplement. Our results indicate that the different pollens have antiviral activity and improve the survival of bees infected with the deformed wing virus variant A. However, the bee pollen from ulmo tree showed the best results in survival and antiviral activity.

### OP-108

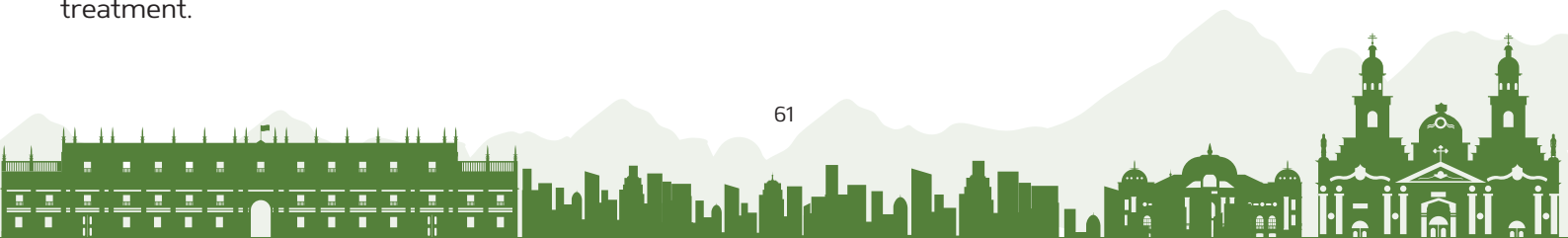
#### Resistance to pyrethroids in *Varroa destructor*: dynamics and perspective

Joel González-Cabrera<sup>1</sup>, Anabel Millán-Leiva<sup>1</sup>, Sara Moreno Martí<sup>1</sup>, Carmen Sara Hernández Rodríguez<sup>2</sup>

<sup>1</sup>Dpto. de Genética, Instituto BIOTECMED. Universitat de València. Burjassot, España.

<sup>2</sup>Dpto. Microbiología y Ecología, Instituto BIOTECMED. Universitat de València. Burjassot, España

*Varroa destructor* is an ectoparasite mite that has proved a devastating stressor that threatens the viability of apiculture worldwide. Parasitized colonies collapse shortly after parasitism in absence of effective control measures. Pyrethroids were reliable for removing mites from the colonies for long time but the evolution of resistance in many locations has limited their use. However, they might still be a useful alternative to other acaricides as part of an integrated management strategy. In our investigations, we have found that resistance to pyrethroids in *V. destructor* is associated with mutations in the Voltage-Gated Sodium Channel, the target of these group of pesticides. A substitution of leucine for valine at position 925 of the channel (L925V) was the first mutation identified as associated with the resistant phenotype in mites collected in Europe. On the other hand, substitutions to methionine and isoleucine at the same position (L925M and L925I) were associated with resistance in the USA but afterwards, they have also been detected in mites collected in Europe. Finally, a new mutation, but this time at position 918 (M918L) were detected in mites already positive for mutation L925V. A thorough analysis of sequencing data showed that mutations L925V and L925M evolved independently in Europe and the USA, respectively. Then, subsequent events have generated the double mutants M918L/L925V in Europe and the L925I mutants in the USA. All the information generated so far have been used for designing diagnostic methodologies, able to accurately differentiate between resistant and susceptible mites. We have used them to assess the frequency of resistance in a vast number of samples. The data show highly heterogeneous patterns, with samples containing a high number of resistant mites and others with very low frequency of them. This, along with evidence of reduced fitness observed in resistant mites, allows considering pyrethroids as a viable alternative in acaricide rotation programs. However, it is essential to carry out a prophylactic diagnostic to evaluate the level of resistance present in a particular apiary before implementing the treatment.





### OP-109

## Reduced efficacy of amitraz for controlling *Varroa destructor* is associated with mutations in an octopamine receptor

C. Sara Hernández Rodríguez<sup>1</sup>, Sara Moreno Martí<sup>2</sup>, Gabrielle Almecija<sup>3</sup>, Krisztina Christmon<sup>4</sup>, Josephine D. Johnson<sup>5</sup>, Marie Ventelon<sup>6</sup>, Dennis Vanengelsdorp<sup>4</sup>, Steven Cook<sup>5</sup>, Frank D. Rinkevich<sup>7</sup>, Rassol Bahreini<sup>8</sup>, Joel Gonzalez Cabrera<sup>2</sup>

<sup>1</sup>Instituto BIOTECMED & Dpto. de Microbiología y Ecología. Universitat de València, Spain

<sup>2</sup>Instituto BIOTECMED & Dpto. de Genética. Universitat de València, Spain

<sup>3</sup>APINOV, Centre de Recherche et Formation Apicole, France & Institut de Recherche sur la Biologie de l'Insecte. Université de Tours, France

<sup>4</sup>Department of Entomology, University of Maryland, MD, USA

<sup>5</sup>USDA-ARS Bee Research Laboratory. MD, USA

<sup>6</sup>Association pour le Développement de l'Apiculture en Auvergne Rhône Alpes (ADA AURA). France

<sup>7</sup>USDA-ARS Honey Bee Breeding, Genetics, and Physiology Laboratory. LA, USA

<sup>8</sup>Department of Biological Sciences, University of Alberta, Canada

Varroosis is the leading cause of colony losses in honeybees (*Apis mellifera*). It is caused by *Varroa destructor*, a mite that parasitizes bees throughout their biological cycle. Controlling the presence of this parasite is essential to prevent colony decline, but beekeepers have limited options apart from the use of synthetic acaricides. The intensive use of these acaricides has contributed to the emergence of resistance in mite populations.

Currently, amitraz is the most widely used acaricide to control *V. destructor*. However, its efficacy has already been compromised in some countries, including France, the United States, Canada, and Spain. To understand the mechanism of amitraz resistance, we identified and characterized the octopamine and tyramine receptors (amitraz targets) of *V. destructor*. By comparing the sequences obtained from mites collected in apiaries with different treatments, we found that different amino acid substitutions in one of the octopamine receptors (Oct $\beta_2$ R) were associated with treatment failures in several countries. Furthermore, these mutations varied depending on the geographical location of the mite colonies.

This finding allowed us to develop and test three diagnostic assays based on the TaqMan<sup>®</sup> technique, which accurately identify mites with mutations in this receptor. This is crucial information for beekeepers, as it can help them select the most appropriate treatment for their apiary based on the mites' susceptibility to acaricides.

### OP-110

## Impact of prolonged acaricidal treatments on *Varroa destructor* resistance

Sara Moreno Martí<sup>1</sup>, Anabel Millán Leiva<sup>1</sup>, Enrique Simó<sup>2</sup>, Inmaculada Segura<sup>3</sup>, Ana Mompó<sup>3</sup>, María José Mahiques<sup>3</sup>, Fernando Calatayud<sup>2</sup>, Joel González Cabrera<sup>1</sup>, Carmen Sara Henández Rodríguez<sup>4</sup>

<sup>1</sup>Instituto BIOTECMED & Dpto. de Genética. Universitat de València, Spain

<sup>2</sup>Agrupación de Defensa Sanitaria Apícola APIADS. Montroi, Spain

<sup>3</sup>Agrupación de Defensa Sanitaria Apícola APICAL y APIVAL. Muro de Alcoy, Spain

<sup>4</sup>Instituto BIOTECMED & Dpto. de Microbiología y Ecología. Universitat de València, Spain

*Varroa destructor* is a parasitic mite that poses a serious threat on honeybee colonies worldwide. Its control has proved challenging for beekeepers since there are few alternatives to remove the mites from the colonies. Pyrethroids, organophosphates, and amidines have been used with success for years but the evolution of resistance in *V. destructor* populations is now a common scenario that jeopardizes the development of apiculture in many locations.

This study was conducted to analyze the effect of commercial acaricides application on *V. destructor* resistance evolution in two independent experimental apiaries. The hives were categorized into three treatment groups: Apistan<sup>®</sup> (active ingredient (a.i.) tau-fluvalinate, pyrethroid), Checkmite<sup>®</sup> (a.i. coumaphos, organophosphate), and Apitraz<sup>®</sup> (a.i. amitraz, formamidine). Additionally, an oxalic acid treated control group was included in the study to prevent colony loss. Bioassays and molecular diagnosis were used to evaluate the resistance levels to each acaricide at three time points (before the first treatment, one year and two years after the initial treatment).

The results showed the impact of the prolonged use of the same acaricide on the resistance evolution within each group. These findings can help to develop better control strategies for *V. destructor* avoiding the emergence of multiresistant mite populations.



## OP-111 Hazardous agricultural pesticides used in Latin America

Grecia Stefanía de Groot<sup>1</sup>, Patricia Sanchez-Aldea<sup>2</sup>, Marcelo Adrián Aizen<sup>3</sup>, Karina Antúnez<sup>13</sup>, Natalia Arbulo<sup>15</sup>, Marina Basualdo<sup>4</sup>, Belén Branchiccela<sup>5</sup>, Adriana Correa Benitez<sup>6</sup>, E. Y. Gutiérrez-Gamiño<sup>6</sup>, Pablo Juri<sup>7</sup>, Silvia Martínez<sup>8</sup>, Gonzalo A. R. Molina<sup>9</sup>, Carolina Laura Morales<sup>1</sup>, Diana Pimentel Betancurt<sup>14</sup>, Jaime Rodríguez<sup>16</sup>, Natalie Herrera Salazar<sup>16</sup>, Sheena Salvarrey<sup>10</sup>, Carlos Melo Silva-Neto<sup>11</sup>, Gherardo Bogo<sup>12</sup>

<sup>1</sup>ECOPOLO Grupo de Investigación en Ecología de la Polinización, Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA, CONICET-Universidad Nacional del Comahue), San Carlos de Bariloche, Argentina, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>2</sup>Facultad de Ciencias de La Salud, Universidad SEK, Instituto de Investigación Interdisciplinaria en Ciencias Biomédicas SEK, Santiago, Chile, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>3</sup>ECOPOLO Grupo de Investigación en Ecología de la Polinización, Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA, CONICET-Universidad Nacional del Comahue), San Carlos de Bariloche, Argentina

<sup>4</sup>Universidad Nacional del Centro de la Provincia de Buenos Aires, Facultad de Ciencias Veterinarias, PROANVET, Tandil, Buenos Aires, Argentina, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>5</sup>Sección Apicultura. Instituto Nacional de Investigación Agropecuaria. Ruta 50 m 11. Colonia, Uruguay, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>6</sup>Departamento de Medicina y Zootecnia de Abejas, Conejos y Organismos Acuáticos (DMZ:ACyOA), Facultad de Medicina Veterinaria y Zootecnia (FMVZ), Universidad Nacional Autónoma de México (UNAM), Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>7</sup>Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>8</sup>Área Apicultura, Subsecretaría de Agricultura, Ministerio de Producción y Agroindustria de Río Negro. Argentina, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>9</sup>Facultad de Agronomía, Universidad de Buenos Aires, Departamento de Producción Animal. Ciudad Autónoma de Buenos Aires, Argentina, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>10</sup>Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>11</sup>Federal Institute of Education, Science and Technology of Goiás, Brazil. 74594-111, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>12</sup>ECOPOLO Grupo de Investigación en Ecología de la Polinización, Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA, CONICET-Universidad Nacional del Comahue), San Carlos de Bariloche, Argentina, CREA Research Centre for Agriculture and Environment, Bologna, Italy, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

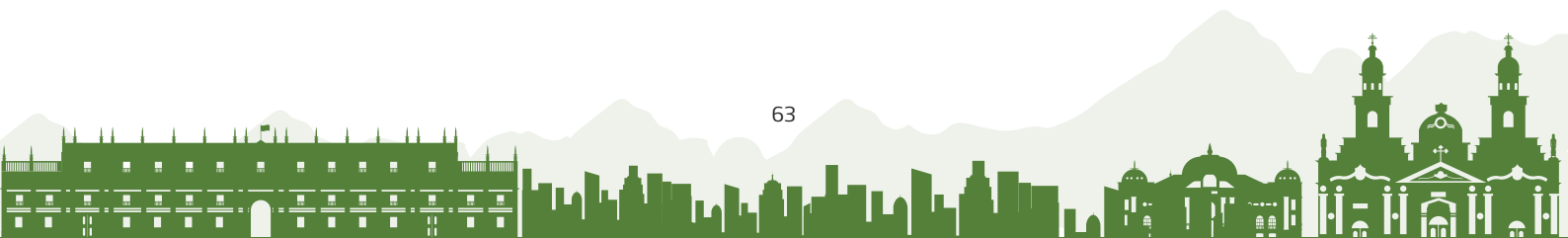
<sup>13</sup>Laboratorio de Microbiología y Salud de las Abejas. Departamento de Microbiología. Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>14</sup>Teagasc Food Research Centre, Moorepark, Fermoy, County Cork, Ireland, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>15</sup>Centro Universitario Regional del Este, Universidad de la República. Sede Rocha. Rocha, Uruguay, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>16</sup>Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

During the last 70 years, modern agriculture has transformed food production globally, increasing the demand of external synthetic inputs. Particularly, the volume of pesticides used in Latin America (LA) has increased 500 % over the last 30 years. However, in Europe -the region with the strictest regulatory system to protect biodiversity and promote food security- pesticide use has barely increased 3 % in the same period. Considering these contrasting trends in the use of pesticides, we hypothesize that the identity and hazardousness of their active ingredients (AI) also reflect unequal development of environmental policies between these two regions in the last decades. Pesticides used in agriculture pose serious threat for bees; therefore, knowledge regarding their identity and hazardousness is relevant for beekeeping and pollination services. In this study, we registered all the AI approved for use in eight LA countries over 10 economically important crops and recorded their target organism and legal status in the European Union (EU). We explored the variance in the total number of approved AI as well as in the number of unapproved AI in EU, using Generalized Linear Mixed Models with country and crop as random variables. A total of 523 AI approved in eight LA countries over 10 crops were identified and approximately 50% were unapproved in the EU. Regarding target organism, 202 AI were herbicides, 157 target animals (e.g., insects, rodents), 147 were fungicides, with the remaining 17 being multi-target. Statistical analyses showed that 30 % of the variability in the number of AI approved and 39 % in AI unapproved in the EU are related to country and crop identity, indicating particularities but also a general pattern for the entire region. Findings indicate implementation of a weaker regulatory framework for hazardous pesticides that reflect a more permissive environmental policies in LA compared to the EU. Considering the increasing global demand of food and the importance of LA in their supply, these results should raise an alert about the consequences of pesticide use on ecosystems.



### OP-112

## results of the monitoring of the biological relationship of Africanized honeybees with varroa since 2002 in two geographic zones in Santa Cruz, Bolivia

Nilo Padilla Osinaga

No affiliation

Varroa has become the number one threat to beekeeping in the world. The solution to this problem has been the control of varroa with sanitary treatments, but today we see that this solution has further complicated the possibility of a sustainable solution, leading the hives to a state of dependence and the varroa to a state of resistance. In our Africanized region, since 2000 we have been monitoring the varroa bee relationship, focusing on two factors, the hygienic behavior of the bees and the variations of varroa populations in the hives, the results show that our bees and varroa have developed strategies of coexistence, These results were published in the different beekeeping conferences of ADAPICRUZ (Association of Beekeepers of the Department of Santa Cruz, Bolivia). It is a fact that Africanized bees have populated the entire tropical zone of America from northern Argentina to Mexico, including southern Bolivia, in this area of our country the results are different, the bees have a high degree of dependence on health controls, this shows that the factors of coevolution varroa bees have varied despite having the same genetic origin and the same environment, the differentiating factor is the management techniques and especially having made preventive treatments in a sustained manner, without strategic objective. The control of pests and diseases in beehives positioned in the minds of producers continues to advance stealthily, putting at serious risk the results obtained in our production system based on the biological balance of bees with their threats, in solutions based on nature and without sanitary control. It is urgent to make an objective analysis, we all know that the only way out of this situation is through achieving a relationship of coexistence of varroa bees that gives viability to beekeeping as a productive sector and the survival of bees in the medium and long term.

### OP-113

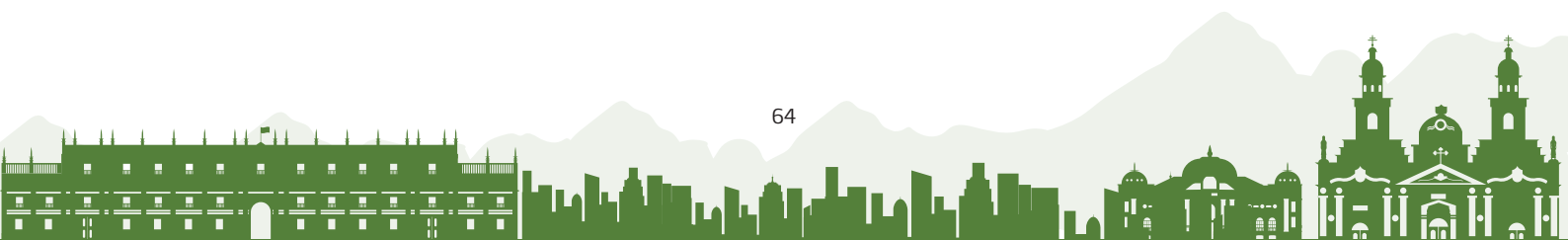
## Selection of chemical insecticides to control the invaded honey bee pest, *Vespa velutina*

Donguei Hong<sup>1</sup>, Chuleui Jung<sup>2</sup>

<sup>1</sup>Department of Plant Medicals, Andong National University, Korea

<sup>2</sup>Agriculture Science and Technology Research Institute, Andong National University, Korea

In the method of controlling *Vespa* using chemicals, coating insecticides on *Vespa* visiting apiary and returning them to their nests. Returned worker can kill the entire nest by spreading insecticides to adults and larvae in the nest. Although many beekeepers in Korea use these control methods, standards and effects have not been established. In this study, insecticides used by beekeepers in Korea were searched and selected, and the control effect was verified to apply them to *Vespa velutina*, an invasive species. Eight insecticides (Clothianidin, Bifenthrin, Carbosulfan, Dinotefuran, Cartap hydrate, Carbaryl, Dinotefuran, Etofenprox, etc.) were tested on adult worker and larvae. Adults were tested for local contact toxicity each 50 $\mu$ l. And larvae were tested to oral toxicity by feeding each 50 $\mu$ l, 3 times. As a result of the adult experiment, more than 70% of Clothianidine, Dinotepuran, and Carbosulfan died within 30 minutes after contact with the insecticides and showed rapid efficacy. Carvaryl followed, Bipenthrin and Cartap hydrochloride were relatively low-toxic. As a result of Clothianidine, Dinotepurane, and Carbosulfan by concentration, median lethal dose (LD50) per individual is Clothianidine 0.29, Dinotefuran 0.65, and Carbosulfan 4.41 $\mu$ g. It were higher than honeybees 0.044, 0.023, and 0.18 $\mu$ g. In case of larvae, it was fed 3 times every 12 hours, but death began after second treatments. After 3rd treatments (72h), all insecticides showed a mortality more than 70%. The median lethal time (LT50) was the fastest at about Cabaryl 34, Clothianidine 40, and Dinotefuran 43h. And it was relatively slow at Bifenthrin 86 and Cartap hydrochloride 73h. The toxicity to larvae tended to be similar to that of adults. When applying the results of this study, flight speed, distance, and topography should be considered. Since larvae need to be exposed to more insecticides than adults to die, it would be effective to treat multiple times. *Vespa velutina*'s chemical susceptibility was similar to that of honeybees. Therefore pollution and residual problems with non-target organisms and the environment, including honeybees is should be considered carefully.



### OP-114

## The *Vespa orientalis* Linnaeus 1771 (Hymenoptera: Vespidae) issue in Chile: The urgency of taking control measures through collaboration between beekeepers, specialists, and the government

José Contreras

Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile

The *Vespa orientalis* Linnaeus 1771 (Hymenoptera: Vespidae), also known as the Asian hornet, is a eusocial wasp native to the Northern Hemisphere and mainly distributed in Eurasia and North Africa. It is a well-known species in countries such as Malta, Spain, Italy, and Israel, where it causes significant losses to beekeeping and fruit cultivation, and can cause complex symptoms, including renal failure and even death, particularly in children.

In May 2019, the species was first recorded in Santiago, Chile, and the first scientific record was published in 2020, including specimens collected outside a nest in the San Bernardo commune of the Metropolitan Region of Santiago. To date, the species has been recorded in multiple communes in the Metropolitan Region, with at least two nests collected, cases of human stings reported, and recent sightings in at least three locations in the O'Higgins region.

Given the lack of government measures for monitoring and managing this species in Chile, we aim to provide an update on its distribution in Chile and provide guidelines for joint actions among specialists, beekeepers, and government institutions (SAG, INIA, and MINSAL). This will be based on scientific evidence and the experiences of international specialists and beekeepers.

Our study includes new distribution records, physical nest collections, conversations with beekeepers, and collaboration with international specialists who have experience in controlling this species. The evidence collected supports the urgency of implementing control measures and the need for collaborative actions among stakeholders to minimize the negative impact of this invasive species on the environment, beekeeping, and public health.

In summary, our study highlights the urgent need for measures to control the spread of *Vespa orientalis* in Chile and the importance of collaboration among specialists, beekeepers, and government institutions to address this issue.

### OP-115

## Beekeeping in Cuba: Selection, Resistance, and Diseases

Adolfo Pérez Piñeiro<sup>1</sup>, Anais Rodríguez<sup>1</sup>, Carlos Ariel Yadró<sup>1</sup>, Alejandro Pérez Morfi<sup>1</sup>, Carlos Enrique Rodríguez Rosell<sup>1</sup>, Ivanna H Tomasco<sup>2</sup>, Karina Antúnez<sup>3</sup>, Belén Branchiccela<sup>4</sup>, Ciro Invernizzi<sup>5</sup>, Isobel Grindrod<sup>6</sup>, Georgiana Webb<sup>6</sup>, Stephen Martin<sup>6</sup>

<sup>1</sup>Centro de Investigaciones Apícolas, La Habana, Cuba

<sup>2</sup>Departamento de Ecología y Evolución, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

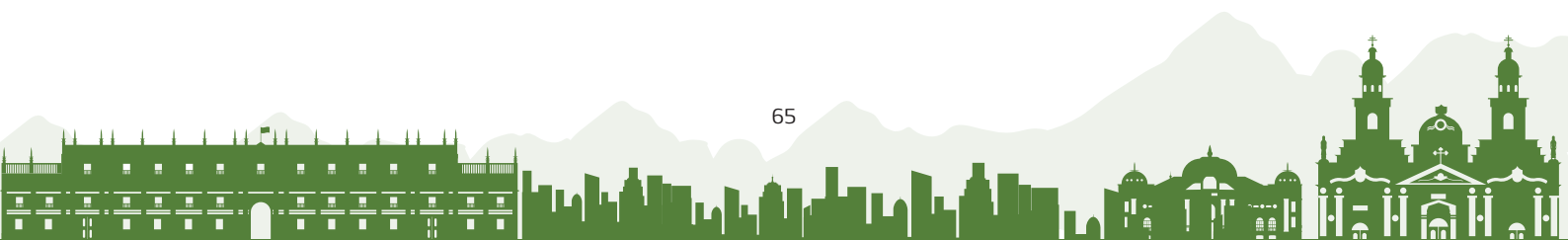
<sup>3</sup>Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

<sup>4</sup>Sección Apicultura, Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay

<sup>5</sup>Sección Etología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

<sup>6</sup>University of Salford, Manchester M5 4WT, UK

Since the beginning of the 80s of the 20th century, Cuba has worked on the selection of bees with the aim of achieving greater honey productivity and resistance to infectious diseases and *Varroa*. However, it wasn't until 2017 that the genetic composition of the Cuban honey bee population was evaluated, and a diagnosis of its main diseases was made using molecular biology tools. These investigations demonstrated that the populations of *Apis mellifera* in Cuba have a European origin (80% similarity with mitochondrial haplotypes), but with differences due to genetic isolation. In addition to the Korean haplotype of *Varroa destructor*, we also detected the presence of *Nosema ceranae*, Acute bee paralysis virus (ABPV), Deformed wing virus (DWW) and Sacbrood bee virus (SBV) in the Cuban colonies. Nevertheless, there have been no reports of hive deaths due to these bee diseases circulating in the country. Said genetic isolation combined with an acaricide-free beekeeping have possibly influenced the fixation and expression of *Varroa*-resistance genes, leading to the high rates of recapping of *Varroa*-infested worker cells (72 %), high removal of mites (81 %) and corresponding low mite fertility ( $r = 0.77$ ), that we also found in Cuban colonies.



### OP-116

## Using the Unhealthy Brood Odor (UBO) assay, a novel hygiene-eliciting assay, as a selection tool in bee breeding programs

Samantha A. Alger<sup>1</sup>, M. Sydney Miller<sup>1</sup>, P. Alexander Burnham<sup>2</sup>, Kaira Wagoner<sup>3</sup>, Micheal Palmer<sup>4</sup>, Adam Collins<sup>5</sup>, Andrew Munkres<sup>6</sup>, Jack Rath<sup>7</sup>, David Peck<sup>8</sup>

<sup>1</sup>Department of Plant and Soil Science, University of Vermont, Burlington, USA

<sup>2</sup>Department of Biology, University of Vermont, Burlington, USA

<sup>3</sup>Department of Biology, University of North Carolina Greensboro, Greensboro, USA

<sup>4</sup>French Hill Apiaries, Saint Albans, USA

<sup>5</sup>French Hill Apiaries, Saint Albans, USA, Vermont Bees, Swanton, USA

<sup>6</sup>Lemon Fair Honeyworks, Cornwall, USA

<sup>7</sup>Hundred Acre Wood Apiary, Pawlet, USA

<sup>8</sup>Betterbee, Greenwich, USA

Vermont USA is home to several well-known bee producers whose bees are prized in the region due to their adaptiveness to the northeast climate. In 2021, the University of Vermont Bee Lab began leveraging diagnostic services for Vermont bee producers towards the development of more disease resistant stock. In 2022, we began to trial the Unhealthy Brood Odor (UBO) assay across three major bee producers with long-time selection programs. UBO is a novel tool to assess a colony's performance of hygienic behavior. The assay measures a colony's hygienic response to a blend of synthetic pheromones that simulate unhealthy brood. UBO is anticipated to be available to a broader market by Spring 2024. In preparation for its release, we present case study examples of three Vermont breeding operations that are using UBO to identify and select for stock that is more disease and pest resistant. We share results of this new assay in the context of both open mated and instrumentally inseminated programs. By pairing pest and pathogen sampling with UBO scores, we show how UBO performance correlates with lower pest and pathogen loads. In particular, we found that high UBO performers have reduced *Varroa* and *Vairimorpha* (Nosema) loads. We share lessons learned and how beekeepers are selecting for these traits in their operations.

### OP-117

## Summer treatment with Aluen CAP™ limited the growth of *Varroa destructor* infestations

Dan Aurell<sup>1</sup>, Selina Bruckner<sup>1</sup>, Lewis Bartlett<sup>2</sup>, Jennifer Berry<sup>3</sup>, Steven Cook<sup>4</sup>, Geoffrey R. Williams<sup>1</sup>

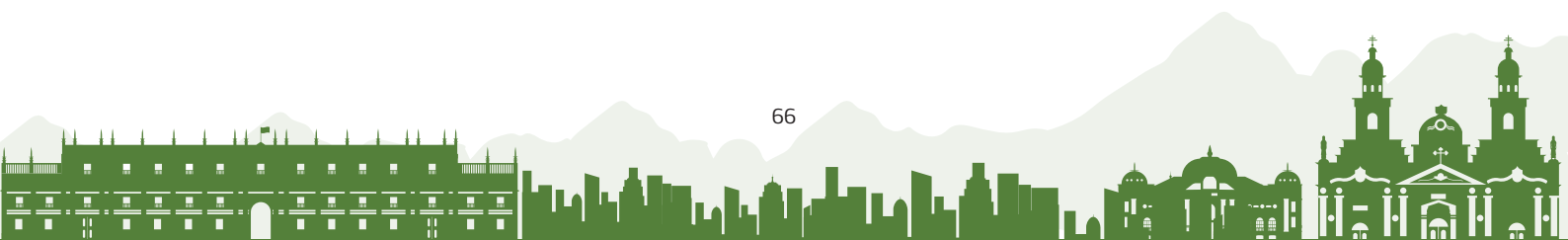
<sup>1</sup>Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA

<sup>2</sup>Center for the Ecology of Infectious Diseases, Odum School of Ecology, University of Georgia, Athens, GA, USA

<sup>3</sup>Department of Entomology, University of Georgia, Athens, GA, USA

<sup>4</sup>USDA-ARS, Bee Research Laboratory, Beltsville, MD, USA

Beekeepers continue to suffer from the effects of *Varroa* mite (*Varroa destructor*) parasitism of their honey bee (*Apis mellifera*) colonies. While effective *Varroa* treatments exist, each product has limitations to its use, and *Varroa* mites continue to develop resistance to treatments. Beekeepers need additional *Varroa* treatments that are safe and effective across a range of conditions, specifically when temperatures are high, brood is present, and honey production is ongoing (e.g., during summer). We predicted that Aluen CAP™, a slow-release oxalic acid formulation developed in Argentina, could fill this gap for beekeepers in the United States of America. We performed the first large-scale U.S. field trial to determine the effects of Aluen CAP™ on *Varroa* mites and honey bee colonies, during summer 2021 in three U.S. states (Alabama, Maryland and Georgia). We applied four strips of Aluen CAP in colonies in two Deep Langstroth brood chambers (and honey supers as needed); the treatment lasted 42 days. Our preliminary analysis of post-treatment *Varroa* infestation of adult bees showed that Aluen CAP™ had an efficacy of 54.9% (34.6–68.9; 95% CI) against *Varroa*, relative to no treatment. Overall, we found that *Varroa* mite infestations rose during treatment with Aluen CAP™, but did not rise as much as in untreated control colonies. In one trial location, *Varroa* infestations remained similar during treatment with Aluen CAP™, but in the two other locations *Varroa* infestations rose during treatment. Samples of broodnest honey were also taken before and after treatment: Initial tests showed a slight increase in oxalate content of honey in Aluen CAP™ treated colonies relative to controls. Our results suggest that Aluen CAP™ could substantially benefit honey bee health, if it is applied as a supplemental treatment during periods that are unfavorable for existing *Varroa* treatments. However, the treatment did not reduce *Varroa* infestations in our conditions, which is important when *Varroa* infestations have reached damaging levels.





### OP-118

## Colony losses and renewal in the United States: Trends in losses, new queens, and new colonies from five years of the Bee Informed Partnership's national survey

Dan Aurell<sup>1</sup>, Mikayla Wilson<sup>2</sup>, Selina Bruckner<sup>1</sup>, Nathalie Steinhauer<sup>2</sup>, Geoffrey R. Williams<sup>1</sup>

<sup>1</sup>Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA

<sup>2</sup>Department of Entomology, University of Maryland, College Park, MD, USA, Bee Informed Partnership, College Park, MD, USA

The Bee Informed Partnership's Loss and Management Survey has been conducted annually since 2007. Based on results from this survey, we will share the latest trends in honey bee (*Apis mellifera*) colony losses in the United States of America and discuss U.S. beekeepers' management practices related to new queens and establishing new colonies. Other studies have shown that beekeepers who replace their queens and who split their colonies tend to have lower winter losses. Even though queen issues are reported as a leading cause of colony death in the U.S., we have not learned in detail what specific signs beekeepers are observing, or what practices they are using in this important area. A preliminary analysis of three years of data showed that only 55% of backyard beekeepers (i.e., those managing 50 or fewer colonies) reported splitting their colonies, whereas 93% and 91% of sideline and commercial beekeepers did (i.e., those managing 51-500 and more than 500 colonies, respectively). Because of the profound differences in management practices between small- and large-scale beekeepers and because the U.S. is large and geographically diverse, we also expect to see effects of operation size and geographic location on variables such as: the kinds of queen issues reported; the proportion of colonies that are requeened each year; whether mated queens or queen cells are used; and on what calendar date beekeepers begin splitting their colonies. The objective of this study is to determine the prevalence of different practices among groups of U.S. beekeepers to reveal opportunities for particular groups of beekeepers to optimize their management.

### OP-119

## Use of statistical models to predict the interspecific transmission of pathogenic microorganisms in wild bees in Italy

Giovanni Cilia<sup>1</sup>, Rossella Tiritelli<sup>2</sup>, Simone Flaminio<sup>3</sup>, Laura Zavatta<sup>1</sup>, Rosa Ranalli<sup>4</sup>, Donato Antonio Grasso<sup>2</sup>, Stefano Leonardi<sup>2</sup>, Marino Quaranta<sup>1</sup>, Antonio Nanetti<sup>1</sup>, Laura Bortolotti<sup>1</sup>

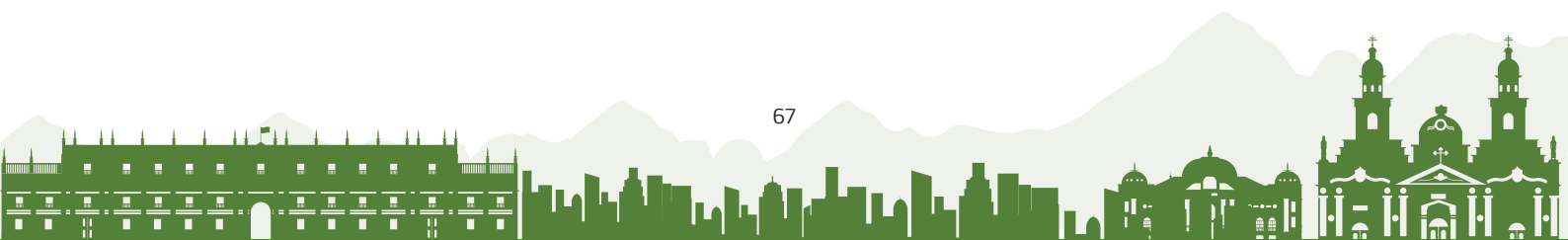
<sup>1</sup>CREA Research Centre for Agriculture and Environment, Bologna, Italy

<sup>2</sup>Department of Chemistry, Life Sciences & Environmental Sustainability, University of Parma, Parma, Italy

<sup>3</sup>Zoology Lab, University of Mons, Mons, Belgium

<sup>4</sup>ZooPlantLab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy

Beekeepers continue to suffer from the effects of Varroa mite (*Varroa destructor*) parasitism of their honey bee (*Apis mellifera*) colonies. While effective Varroa treatments exist, each product has limitations to its use, and Varroa mites continue to develop resistance to treatments. Beekeepers need additional Varroa treatments that are safe and effective across a range of conditions, specifically when temperatures are high, brood is present, and honey production is ongoing (e.g., during summer). We predicted that Aluen CAP™, a slow-release oxalic acid formulation developed in Argentina, could fill this gap for beekeepers in the United States of America. We performed the first large-scale U.S. field trial to determine the effects of Aluen CAP™ on Varroa mites and honey bee colonies, during summer 2021 in three U.S. states (Alabama, Maryland and Georgia). We applied four strips of Aluen CAP in colonies in two Deep Langstroth brood chambers (and honey supers as needed); the treatment lasted 42 days. Our preliminary analysis of post-treatment Varroa infestation of adult bees showed that Aluen CAP™ had an efficacy of 54.9% (34.6-68.9; 95% CI) against Varroa, relative to no treatment. Overall, we found that Varroa mite infestations rose during treatment with Aluen CAP™, but did not rise as much as in untreated control colonies. In one trial location, Varroa infestations remained similar during treatment with Aluen CAP™, but in the two other locations Varroa infestations rose during treatment. Samples of broodnest honey were also taken before and after treatment: Initial tests showed a slight increase in oxalate content of honey in Aluen CAP™ treated colonies relative to controls. Our results suggest that Aluen CAP™ could substantially benefit honey bee health, if it is applied as a supplemental treatment during periods that are unfavorable for existing Varroa treatments. However, the treatment did not reduce Varroa infestations in our conditions, which is important when Varroa infestations have reached damaging levels.



### OP-120

## Assessing the detoxification effect of curcumin and rosmarinic acid on colonies exposed to flupyradifurone and carbaryl in the field

Yongrak Kang<sup>1</sup>, Saeed Mohamadzade Namin<sup>2</sup>, Chuleui Jung<sup>3</sup>

<sup>1</sup>Department of Plant Medicinals, Andong National University, Republic of Korea

<sup>2</sup>Agricultural Science and Technology Institute, Andong National University, Republic of Korea

<sup>3</sup>affiliation 1. Department of Plant Medicinals, Andong National University, Republic of Korea, affiliation 2. Agricultural Science and Technology Institute, Andong National University, Republic of Korea

Honeybees are responsible for pollinating approximately one-third of the world's crop species, but their populations are declining due to exposure to pesticides through various routes. Turmeric and Rosemary have been found to exhibit several biological activities including anti-oxidant, anti-inflammatory and anti-microbial properties. These characteristics are attributed to the presence of Curcumin (CU) and Rosmarinic acid (RA) and This research were conducted to evaluate the effect of CU and RA on colony strength factors of honeybee colonies exposed to recommended concentrations of flupyradifurone and carbaryl in two experimental sesame (*Sesamun indicum*) fields. Nine healthy and queen-right colonies with at least 10000 adult bees were used in each field. Each pesticide was applied two times with a 14-day interval. The colony strength factors such as number of workers, brood, honey and pollen area and the weight of colonies were measured on weekly basis starting with the day before pesticide application and ending two weeks after the second pesticide application. The colonies were treated with two liters of CU or RA-supplemented food after pesticide treatment and the control colonies only received sugar solution. The number of adults in honeybee colonies treated with RA increased significantly over time for both pesticides compared to the control group while the number of adults remained constant in a group of colonies fed with CU. Furthermore, as the experiment was conducted in early autumn, the larvae were already in decline; however, the group that was fed with RA showed a significantly lower pace of larval decline compared to the control group. We did not find any significant difference in other colony strength factor between different treatments. This study demonstrated that RA can protect different stages of honeybees in the colonies exposed to carbaryl and flupyradifurone. Further experiments are required to evaluate the effect of RA and CU on honeybees exposed to different classes of insecticides. Additionally, the detoxification mechanism of RA in both immature and adult stages need to be investigated in future studies.

### OP-121

## Effects of chemical and biological alternatives for crop pest management on the behaviour of forager honeybees *Apis mellifera* under laboratory conditions

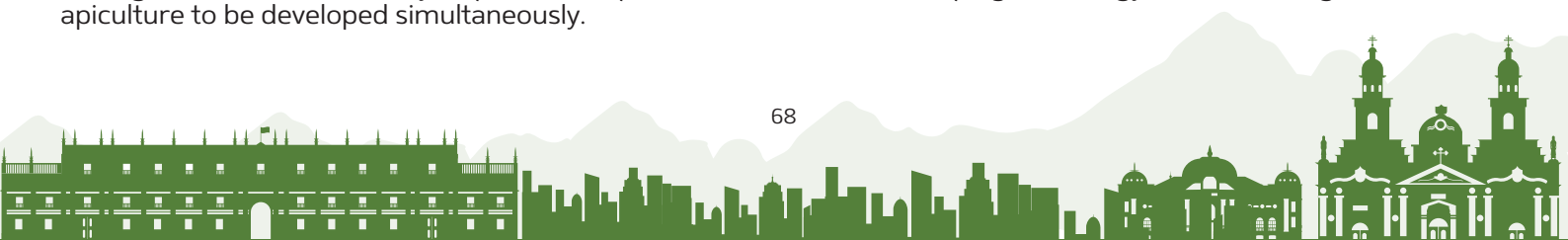
Sandra Milena Aragón Rodríguez<sup>1</sup>, Stephanie Numa<sup>2</sup>, Yuly Paola Sandoval Cáceres<sup>3</sup>, Carolina Diaz Ortiz<sup>3</sup>, Ginna Natalia Cruz Castiblanco<sup>3</sup>, Henry Alexander Bustos Rodriguez<sup>1</sup>

<sup>1</sup>PhD. Researcher, AGROSAVIA, C.I. Tibaitatá. Mosquera, Cundinamarca. Colombia.

<sup>2</sup>M.Sc. Researcher AGROSAVIA, C.I. Tibaitatá. Mosquera, Cundinamarca. Colombia.

<sup>3</sup>B.Sc. AGROSAVIA, C.I. Tibaitatá. Mosquera, Cundinamarca. Colombia.

*Apis mellifera* plays an important role in pollination services for agriculture, nature and also in honeybee byproducts. Nevertheless, since 2016 the number of reportsof affected apiaries has increased in Colombia, possibly related to pesticide exposure of forager bees, consequently leading to a colony collapse. This study aims to compare the selection preference of *Apis mellifera* workers exposed to different concentrations of an active ingredient of biological or chemical pesticides in laboratory conditions. To achieve that, we used a methodology to evaluate the selection preference of a single worker bee *Apis mellifera* in a Y-tube olfactometer where two options were offered: 1) One concentration of the pesticide active ingredient: Thiamethoxam, Imidacloprid, Clothianidin or Fipronil and 2) One of the controls, D-limonene (positive control), clean air (absolute control) or analytic grade acetone (solvent). Each chemical molecule was tested for its lethal dose 50 (LD50), and four additional concentrations ( $\frac{1}{4}$ LD50,  $\frac{1}{2}$ LD50, 2\*LD50 y 4\*LD50). Three biologicals were tested, one based on *Beauveria bassiana*, a granulovirus isolated from *Erynnis ello* pest, and finally a plant pathogen beneficial fungi, *Trichoderma koningiopsis*. For biologicals, the recommended dose (RD) was employed plus four additional concentrations below and above the RD ( $\frac{1}{4}$  RD,  $\frac{1}{2}$  RD, 2\*RD y 4\*RD). We observed that bees responded to all tested chemical molecules, with a direct relation with the concentration where, a higher attraction was given at sublethal concentrations ( $\frac{1}{4}$ LD50 and  $\frac{1}{2}$ LD50), while a rejection behaviour was observed at the LD50, 2\*LD50 and 4\*LD50 doses. The selection response of the worker bees to biological active ingredients was higher for the beneficial fungi *Trichoderma koningiopsis* with no preference for any concentration, while the entomopathogens were not preferred by the insects. These results contribute to understanding individual behaviour in laboratory conditions with single molecules. Nevertheless, further studies must be carried out to unravel the synergistic effect of pesticide mixtures used in agriculture as well as the effect of biologicals as an alternative for pest management in economically important crops, with the aim of developing a strategy that allows agriculture and apiculture to be developed simultaneously.



### OP-122

#### Frequent parasitism of *Apis mellifera* by trypanosomatids in geographically isolated areas with restricted beekeeping movements

Raquel Martín Hernández<sup>1</sup>, Daniel Aguado López<sup>2</sup>, Carolina Bartolomé<sup>3</sup>, Ana Rita Lopes<sup>4</sup>, Dora Henriques<sup>4</sup>, Xulio Masid<sup>3</sup>, Alice Pinto<sup>4</sup>, Mariano Higes<sup>2</sup>

<sup>1</sup>Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA-IRIAF), PCTCLM-INCRECYT (co funded ESF/FSE); Marchamalo, Spain

<sup>2</sup>Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA-IRIAF), Marchamalo, Spain

<sup>3</sup>Grupo de Medicina Xenómica, CIMUS, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Galicia, Spain;

<sup>4</sup>Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Trypanosomatids form a group of high prevalence protozoa that parasitise honey bees, with *Lotmaria passim* as the predominant species worldwide. However, the knowledge about the ecology of trypanosomatids in isolated areas is limited. The Portuguese archipelagos of Madeira and Azores provide an interesting setting to investigate these parasites because of their geographic isolation, and because they harbour honey bee populations devoid of two major enemies: *Varroa destructor* and *Nosema ceranae*. Hence, a total of 661 honey bee colonies from Madeira and the Azores were analysed using different molecular techniques, through which we found a high prevalence of trypanosomatids despite the isolation of these islands. *L. passim* was the predominant species and, in most colonies, was the only one found, even on islands free of *V. destructor* and/or *N. ceranae* with severe restrictions on colony movements to prevent the spread of them. However, islands with *V. destructor* had a significantly higher prevalence of *L. passim* and, conversely, islands with *N. ceranae* had a significantly lower prevalence of the trypanosomatid. *Crithidia bombi* was detected in Madeira and on three islands of the Azores, almost always coincident with *L. passim*. By contrast, *Crithidia mellificae* was not detected in any sample. A High-Throughput Sequencing analysis distinguished two main haplotypes of *L. passim*, which accounted for 98% of the total sequence reads. This work suggests that *L. passim* and *C. bombi* are parasites that have been associated with honey bees predating the spread of *V. destructor* and *N. ceranae*.

This work was funded by the Consejería de Educación, Cultura y Deportes, of the Junta de Castilla – La Mancha (European Regional development Fund) project No. SBPLY/19/180501/000334 and through the program COMPETE 2020—POCI (Programa Operacional para a Competividade e Internacionalização) and FCT (Fundação para a Ciência e a Tecnologia) in the framework of the project BeeHappy (POCI-01-0145-FEDER-029871).

### OP-123

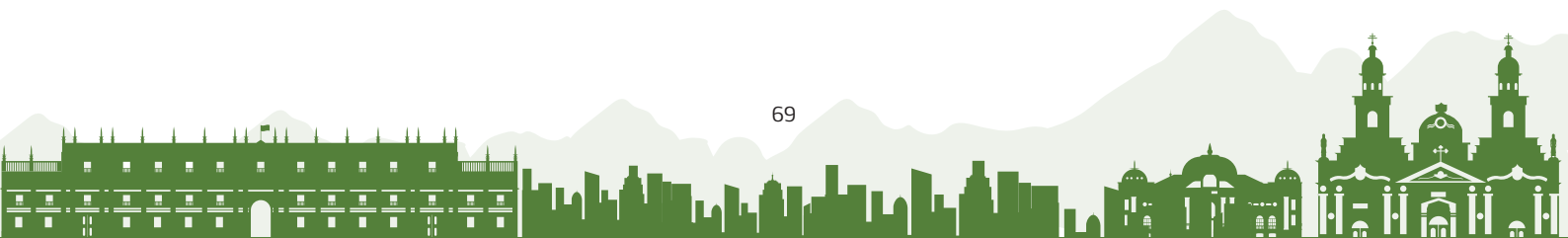
#### Effects of a diet containing 7 probiotic strains (Honeybeeotic™) on commercial honey bee colonies in terms of haemolymph cytology and phenoloxidase activity

Livio Galosi<sup>1</sup>, Evelina Serri<sup>1</sup>, Pierluigi Pierantoni<sup>2</sup>, Silvia Vincenzetti<sup>1</sup>, Sara Berardi<sup>1</sup>, Lucia Biagini<sup>1</sup>, Alessandra Roncarati<sup>1</sup>, Stefania Silvi<sup>1</sup>, Giacomo Rossi<sup>1</sup>

<sup>1</sup>School of Biosciences and Veterinary Medicine, University of Camerino, Matelica, Italy

<sup>2</sup>Cooperative of Mountain Bee-keepers, Matelica, Italy

Starting from 2005, in Roti Abbey area, Matelica, Marche Region, Italy, was identified an honey bee population, isolated from several decades by other populations of *Apis mellifera ligustica*, in an area far from any human activities, without pollutants, and away from "genetic contamination" by other honey bees' populations. We isolated and characterized 7 strains of probiotic bacteria by quantitative PCR along with deep sequencing of amplicons of the V4 region of the bacterial 16S rRNA gene. Through this analysis we observed some important differences regarding both the size and the composition of the microbiome of Roti's bee workers, compared to the common honeybee (*Apis mellifera ligustica*). To investigate the innate immune response of honeybees to microbiota modification, by integration with these 7 probiotic strains of Roti's bees origin (Honeybeeotic™: *Lactiplantibacillus plantarum* DSM 33923; *Apilactobacillus apinorum* DSM 34547; *Lactiplantibacillus fabifermentas* DSM 34546; *Lactiplantibacillus fabifermentas* DSM 34454; *Lactiplantibacillus plantarum* DSM 34542; *Lactiplantibacillus plantarum* DSM 34500, and *Lactiplantibacillus plantarum* DSM 34499), 4 hives received a beekeeper-formulated diets (A = 50% glucose syrup, B = 50% glucose syrup supplemented with probiotics, C = Apifonda®, and D = organic molasses) for one month. The diet B was integrated with the addition of Honeybeeotic™  $2 \times 10^{11}$  bacterial blend daily for 30 days. Phenoloxidase activity and haemolymph cellular subtype count were investigated. Phenoloxidase activity was lowest ( $P < 0.05$ ) in group B ( $5.59 \pm 0.61$ ), with higher activity ( $9.72 \pm 1.54$ ) in group C, and the haemocyte population differed within the four observed groups, with the highest number of oenocytoids ( $\times 8$  cells [4-15]) and granulocytes ( $\times 17$  cells [5-33]) in group C, followed by groups D and A. Although, immune responses following probiotic diet-integration have still not been completely clarified, this investigation demonstrates that, regardless of the type of artificial diet administered, an association between cell immunity and the phenoloxidase activity decrease is observable in bees supplemented with probiotics (Honeybeeotic™) compared to other unintegrated groups of bees. Modulation of the innate immune response of bees through the use of highly specific probiotics could improve feed sustainability and agricultural pollination efficiency by supporting larger, healthier honey bee colonies.



### OP-124

## A novel antifungal formulation “Oleuropeyn-B”, for the treatment of Nosemosis caused by the pathogen *Nosema ceranae* in *Apis mellifera*

Jessica Isabel Martinez Arenas<sup>1</sup>, Jose Duguet<sup>2</sup>, Carlos Fabian Zuñiga<sup>1</sup>, Marcelo Eduardo Henriquez<sup>2</sup>

<sup>1</sup>Centro de Medicina Regenerativa, Universidad del Desarrollo, Santiago, Chile

<sup>2</sup>ApiQuality SpA., Tomé, Chile

*Nosema ceranae* is a microsporidium parasite, one of the most prevalent and pathogenic, that silently affects honey bees, causing a disease called nosemosis. This parasite produces resistant spores and germinates in the midgut of honey bees, extrudes a polar tubule that injects an infective sporoplasm in the host cell epithelium, proliferates, and produces intestinal disorders that shorten honey bee lifespan. Research efforts have been developed to find effective and non-contaminant compounds against *N. ceranae*. In this work, we evaluate the antifungal activity of Oleuropeyn-B a novel formulation based on a non-toxic plant extract against *N. ceranae*. The effectiveness of the treatment has been demonstrated by a reduction in the level of infection of 83.6% under laboratory conditions, at concentrations of 0.5 and 1 g/L without affecting the survival rate. Besides, in-field conditions we reported a reduction of 88 % of the infection level at a concentration of 2.5 g/L, obtaining better antifungal effectiveness in comparison to other commercially available treatments. We observed that the use of Oleuropeyn-B led to an increase in population size and honey production, both parameters associated with colony strength. And a slight upregulation was observed in abaecin and hymenoptaecin, two genes associated with humoral immunity. The reported antifungal activity of Oleuropeyn-B against *N. ceranae*, with a significant control of spore proliferation in worker bees, suggests the promising commercial application use of this product against nosemosis.

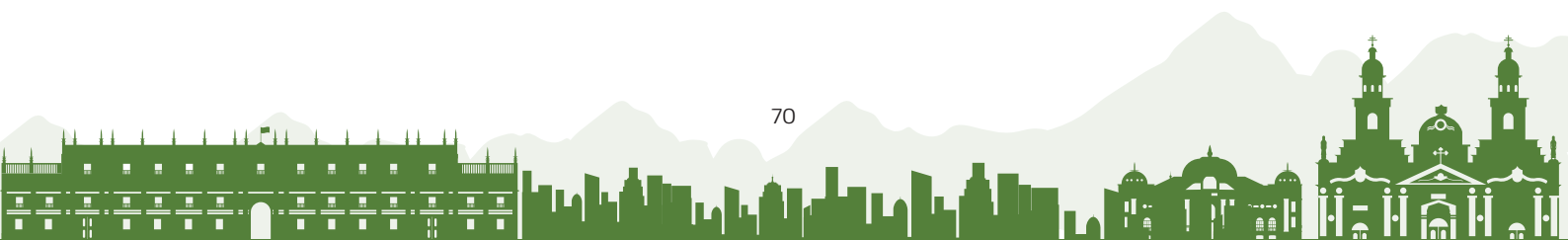
### OP-125

## Disease ecology of a wild population of honey bees (*Apis mellifera*) in south Texas

Myra Dickey, Jordan Twombly Ellis, Mckaela Whilden, Juliana Rangel

Department of Entomology, Texas A&M University

The most detrimental threat to honey bee (*Apis mellifera*) health continues to be the ectoparasitic mite, *Varroa destructor*, which has been linked to colony losses worldwide. *Varroa* is also a prolific vector of several honey bee-associated viruses. In addition, there is also a microsporidian gut pathogen, *Nosema* spp. that has been linked to colony loss. Wild honey bee colonies can be exposed to both of these pathogens. Since wild colonies live in feral conditions, they are more tolerant to *Varroa* parasitization than managed colonies. Thus they might be more tolerant of the associated viral infections *Varroa* transmits. Likewise, since these wild colonies are not treated for *Varroa* or *Nosema* this would allow for natural selection of mite and *Nosema* tolerant bees. To date, there is limited information about virus and *Nosema* prevalence in wild honey bee populations. The Welder Wildlife Refuge (WWR) is a unique site to study the disease landscape of wild bees in the Southern U.S. Our goal is to identify and quantify honey bee-associated viruses in a wild population and compare the presence of these viruses to that in the nearest managed apiaries. We also aim to compare the levels of *Nosema* in this population as it compares to a nearby managed apiary. We have found significant changes of viral titers over time at the WWR for two major viruses. However, there was not a significant difference in viral titers between the managed population and WWR. In addition, we found no differences in the levels of *Nosema* between the wild and managed population. Perhaps indicating that wild honey bees are more tolerant to virus and *Nosema* infections than the managed colony as we do not see the same yearly loss associated with wild colonies.



### OP-126

## The PoshBee legacy: Summary of a 5 year EU-wide project on the effects of pesticides, pathogens and nutrition on bee health

Joachim Rodrigues de Miranda<sup>1</sup>, Jane C Stout<sup>2</sup>, Marie Pierre Chauzat<sup>3</sup>, Marika Mänd<sup>4</sup>, Robert J Paxton<sup>5</sup>, Denis Michéz<sup>6</sup>, Peter Neumann<sup>7</sup>, Alexandra Maria Klein<sup>8</sup>, Francesco Nazzi<sup>9</sup>, Philippe Bulet<sup>10</sup>, Simon G Potts<sup>11</sup>, Teodor Metodiev<sup>12</sup>, Mark Jf Brown<sup>13</sup>

<sup>1</sup>Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>2</sup>School of Natural Sciences, Trinity College Dublin, Dublin, Ireland

<sup>3</sup>Laboratory for Animal Health, ANSES, Paris-Est University, Maisons-Alfort, France

<sup>4</sup>Department of Plant Protection, Estonian University of Life Sciences, Tartu, Estonia

<sup>5</sup>Department of General Zoology, Martin Luther University Halle-Wittenberg, Halle, Germany

<sup>6</sup>Laboratoire de Zoologie, University of Mons, Mons, Belgium

<sup>7</sup>Veterinary and Medical faculty, University of Bern, Bern, Switzerland

<sup>8</sup>Department of Nature Conservation and Landscape Ecology, University of Freiburg, Freiburg, Germany

<sup>9</sup>Department of Agroalimentary, Environmental and Animal Science, University of Udine, Udine, Italy

<sup>10</sup>Platform BioPark Archamps, Archamps, France

<sup>11</sup>Centre for Agroenvironmental Research, University of Reading, Reading, England

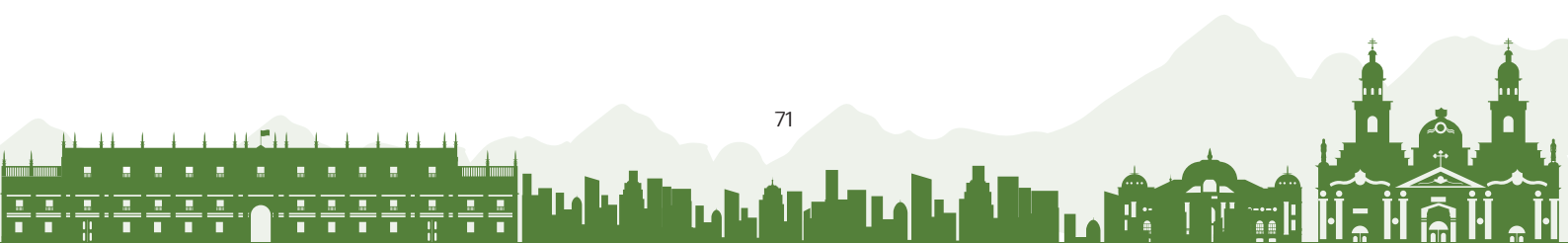
<sup>12</sup>PenSoft Inc. Sofia, Bulgaria

<sup>13</sup>Department of Biology, Royal Holloway University London, London, England

In 2018 a hugely ambitious 5 year EU Horizon 2020 project on the effects of pesticides, pathogens and nutrition on bee health was started. Not only did the project address three classes of agricultural pesticides (insecticides, fungicides and herbicides), multiple pathogens, nutrition and their interactions, but it did so for three different sentinel bee species (honeybees, bumblebees and mason bees) and at multiple scales. The project was organized in 13 work-packages, covering everything from real-world monitoring (WP1, WP2), detailed laboratory (WP3, WP5, WP6) and semi-field experiments (WP7), development of novel tools (WP9) and sentinel bee species (WP4) and prognostic modelling (WP8), as well as a sophisticated multi-layered communication (WP10) and policy strategy (WP11), all coordinated through a well-structured management plan (WP12), and informed by clear legal and ethical guidelines (WP13). The project involved 43 partners from 26 European countries drawn from academia, industry, farmers representatives and beekeeping organizations.

The main conclusions of the project is that, with a few notable exceptions, different stressors interact mostly additively; that nominally inert additives in agrochemical formulations can themselves have significant negative effects on bee health; that adequate nutrition, primarily the quantity and quality of pollen, can compensate for a lot of agrochemical damage; that interactions between stressors are difficult to model using individual agent-based models and that bee haemolymph is a promising matrix for identifying a wide range of markers for bee health.

PoshBee has been very productive, with an enormous output in terms of data, scientific publications, official reports, policy briefings, interviews, conferences, workshops, training, videos and social media presence, through which PoshBee has gained significant recognition and influence. Although PoshBee officially finished in May 2023 it will continue to produce for a while yet, especially through integrated analyses of the cumulative data from the individual work-packages. In this presentation we will summarize PoshBee's main achievements and recommendations.





### OP-127

## The Effects of Mancozeb Fungicide on Survival and Development of Immatures of "*Scaptotrigona bipunctata*"

Jenifer Ramos<sup>1</sup>, Alex Otesbelgue<sup>2</sup>, Luísa Constante Cristmann<sup>1</sup>

<sup>1</sup>Laboratório de Entomologia e Fitopatologia, Embrapa Meio Ambiente, Jaguariuna/São Paulo, Brazil

<sup>2</sup>Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Bees play a crucial role as the main pollinators. In Brazil there are about 240 species of stingless bees that significantly contribute to the maintenance of angiosperm biodiversity and local food production. Given the large agricultural areas and crops in the region, strong investments in pollinator conservation would be expected from the agricultural sector. However, evidence of a decline in pollinator populations, particularly bees, has raised concerns about the potential impact on agricultural productivity and biodiversity. One of the factors contributing to this phenomenon is the overuse of pesticides. Mancozeb, for example, is used in more than ten crops and is the most commercialized fungicide in Brazil. In this scenario, we evaluated the effects of chronic exposure to Mancozeb on the immature stages of the stingless bee *Scaptotrigona bipunctata*. Bees were exposed via diet to five different doses of the fungicide, calculated from the field rate indicated for citrus crops: (I) 1.545 ng; (II) 2.175 ng; (III) 41.29 ng; (IV) 45.71 ng; (V) 960 ng ai/ $\mu$ L of diet), and to non-contaminated food. Each dose had five replicates, with 40 larvae allocated in rearing plates. The deaths were counted every day, and the development of the bees was monitored until they reached the adult stage. To analyze the data, we used Kaplan-Meier and one-way Anova survival curves. The results indicate that even doses considered sublethal to adults of *S. bipunctata* were highly lethal to immature bees, drastically reducing the probability of survival and/or increasing the larval development period. The survival rate was less than 50% in all treatments as: (I) 32% (95% CI: 0.23 - 0.41); (II) 21% (95% CI 0.13 - 0.29); (III) 14% (95% CI: 0.09 - 0.22); (IV) 4% (95% CI: 0.01 - 0.08); and (V) no survival for 960 ng ai/ $\mu$ L. Given the wide use of this fungicide, our findings highlight the need for its re-evaluation and the inclusion of immatures of stingless bee species in toxicological tests for risk assessment.

### OP-128

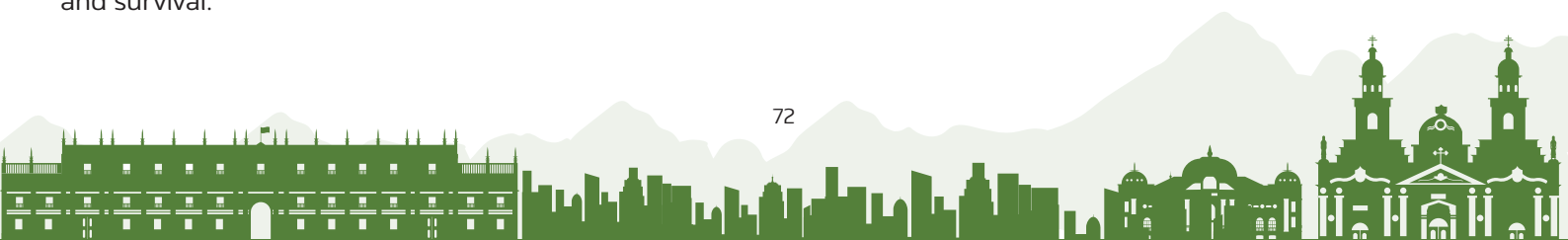
## High incidence of viruses affecting migratory bees of *Apis mellifera* species in Southern Brazil

Matheus Iuri Fröhaufl<sup>1</sup>, Aroni Sattler<sup>2</sup>, Lariane Da Silva Barcelos<sup>1</sup>, Gabriel Da Silva Zani<sup>1</sup>, Nadalin Yandra Botton<sup>1</sup>, Leonardo Classen Ribeiro<sup>1</sup>, Geferson Fischer<sup>1</sup>

<sup>1</sup>Universidade Federal de Pelotas (UFPEL), Pelotas, Rio Grande do Sul, Brazil

<sup>2</sup>Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil

The practice of renting bee hives for migratory pollination is widely used in modern agriculture. Farmers rent hives from beekeepers to enhance crop yields and quality. Unfortunately, this practice has been associated with the spread of diseases and a reduction in wild bee populations. The decline in bee colonies can result from several factors, including pesticide exposure, habitat loss, and the presence of viruses that affect bees. Notably, viruses have been found to substantially impact bee health and survival. This study aimed to evaluate the incidence of the six major viruses that affect migratory honeybees of *Apis mellifera* species in Southern Brazil. Samples were collected from migratory colonies located at the Agronomic Experimental Station - Universidade Federal do Rio Grande do Sul - UFRGS - in Rio Grande do Sul, Brazil. To assess the incidence of the six major viruses affecting the species, namely DWV, BQCV, ABPV, IAPV, SBV, and CBPV, the conventional RT-PCR technique was employed using two multiplexes standardized by the Virology and Immunology Laboratory at Universidade Federal de Pelotas - UFPEL - Brazil. A total of 135 beehives were collected and evaluated for the presence of viruses. Out of the 135 beehives, 12 (8.9%) were positive for ABPV, 124 (91.8%) for BQCV, 85 (63.0%) for CBPV, 123 (91.1%) for DWV, 3 (2.2%) for IAPV, and 96 (71.1%) were positive for SBV. In comparison, a previous study conducted by our group, evaluating the presence of these same viruses in 164 samples of africanized honeybees in 27 apiaries in southern Brazil, found a lower incidence of BQCV (36%), ABPV (3%), and DWV (1%). The higher percentage of virus-infected bees in this study is possibly due to the migratory nature of the bees and various factors that may affect them, such as increased exposure and stress during transportation. These findings suggest that migratory beekeeping may contribute to the spread of viruses in bee populations and highlight the need for further research to better understand the impact of these viruses on bee health and survival.



### OP-129

#### **Ontogeny of individual immunity and virus infection in workers and drones of honey bee (*Apis mellifera*)**

Tal Erez<sup>1</sup>, Tal Erez<sup>2</sup>, Sharif Hamdo<sup>1</sup>, Angelina F. Osabutey<sup>1</sup>, Elad Bonda<sup>1</sup>, Asaf Otmy<sup>1</sup>, Victoria Soroker<sup>1</sup>

<sup>1</sup>Department of Entomology, Agricultural Research Organization, The Volcani Institute, Israel

<sup>2</sup>Department of Entomology, The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University in Jerusalem, Israel

Honey bee colonies are commonly infected by viruses, mainly deformed wing and acute paralysis viruses. As treatment against viruses is highly problematic, breeding for hygienic behavior, one of the major social defense mechanisms, is considered a sustainable way to reduce the impact of Varroa mites and its associated viruses on honey bee health. However, concern has been raised recently that hygienic behavior may facilitate the horizontal transmission of viruses in honey bee colonies. In our study we aimed to evaluate the prevalence and viral loads of the locally most common viruses, during the ontogeny of the workers and drones in high and low hygienic colonies. In addition, the samples of workers and drones tested for viral load were also tested for the expression of immune genes from two immune pathways by RT-PCR. We found differences in the ontogeny of virus infection between drones and workers. In addition, workers and drones also differed significantly in expression of genes from two immune pathways. The significance of these findings will be discussed.

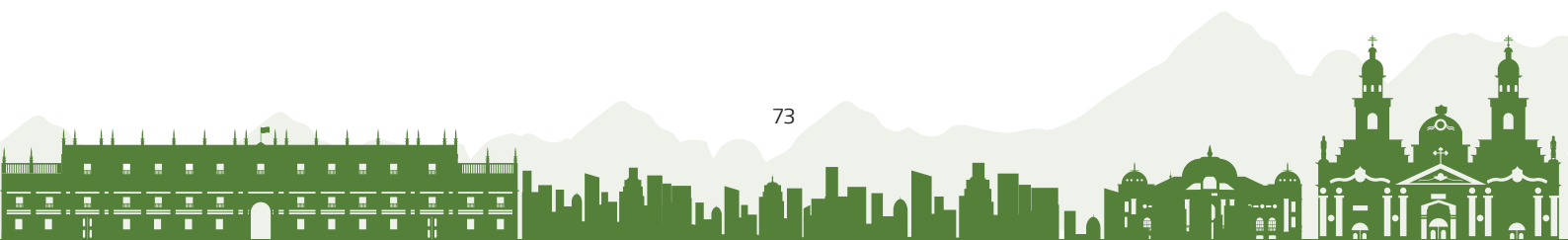
### OP-130

#### **Selective breeding for mite resistance and sustainable management of varroa with natural treatments**

Randy Oliver

ScientificBeekeeping.com

Randy has been performing a simplified "Modified Bond" selective breeding program with 1500 hives for six years. He will present his methods and results to date. He has also run his commercial operation for 21 years using only oxalic and formic acids and thymol for varroa control. He will present some of his recent research on improved application methods for these natural treatments.



## Beekeeping Economy

### OP-131

## The global community of beekeepers overcomes a depressed honey market by restoring authenticity, fair prices and remuneration for producers

Chris Hiatt

American Honey Producers Association

A rising tide raises all boats in the harbor. The United States is the number one importer of honey in the world by far. I will speak on how American Honey Producers Association (AHPA) has increased worldwide standards for honey testing using NMR by getting \$2.5 million appropriated from US Congress to Customs. AHPA is in constant contact with new tech developers who are working to increase the ability of labs to detect syrups, immature honey and resin technology which combats all forms of adulteration. We need this to keep authentic honey producers in business. Partnering with Dr. Michael Roberts (UCLA food fraud center) has brought the story of honey fraud to the forefront within the United Nations, UN FAO, the media and our producer/packer circles in the United States. I will present powerful graphs showing how the AHPA-led anti-dumping suit win in the US International Trade Commission actually helped raise the price of honey for producers in Argentina, Brazil, Vietnam and India. I will cover how we are updating the grading standard and labeling laws for honey within our country, as the country of origin labeling on many bottles is hidden or unreadable. We are combatting Food Fraud with international collaboration. I will touch on how keeping beekeepers in business is a global food security issue for the beekeepers attending Apimondia, due to the importance of food-crop pollination in their own countries. A powerful non-profit in the US is suing and campaigning to get hives off national forest service land and other wild areas so I will cover what AHPA is doing to keep natural land access open to beekeepers. The native bee vs managed bee fight is a waste and drain of resources that is dividing the pollinator community. We are working to keep natural areas accessible for beekeepers. I also look forward to returning to Chile, where I lived for 2 years in the 90's, to reconnect with many old friends.

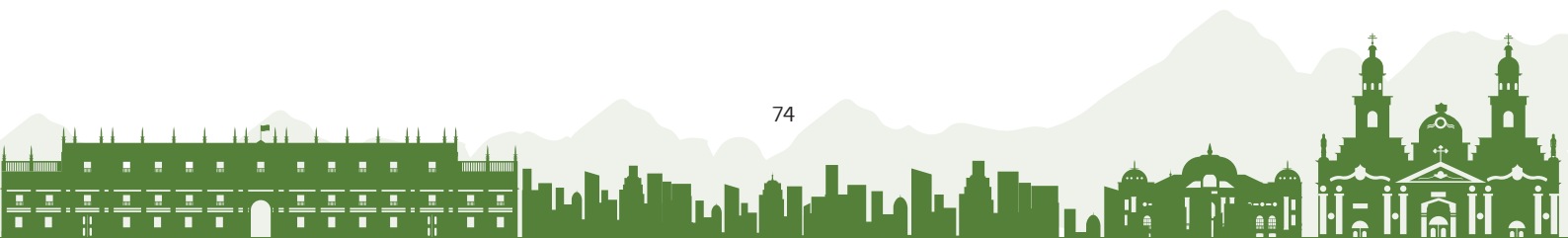
### OP-132

## Cooperative of profesional beekeepers

Ramon Rodriguez Monje

Euromiel, S. Coop. de 2º Grado, Mérida, Badajoz Spain

Our Beekeepers Cooperative is the way to fix poblation in our rural areas. We have more than 550 professional beekeepers with more than 325.000 beehives. We want to explain our experience, in all of areas, purchases and sales, when we buy and sell together, we get the best benefits. We have two pharmacies we give the options for the best health of our beehives. We try to sell with the best quality at the best price for our beekeepers. We only have one estrategy all the thing that are well for us are well for our Customers and suppliers. We want to explain our details to serve as exampel to other beekeepers. Euromiel is a Cooperative of Second degree, formed by three cooperatives of first degree. Apihurdes is in Pinosfranqueado, a village with 1.200 inhabitats. Sierra Miel is in Torrecilla de los Angeles with 708 inhabitants and Montemiel is in Fuenlabrada de los Montes with 2.046 inhabitants. All three villages are more than two hours away from a big cities. We are working together from more than 30 years ago, adapting to the circumtances of each moment. We produce polyflora honey, Forest honey and Chestnut around 3 or 4.000 tons per year. About bee pollen we produce fresh and dry bee pollen 300 tons per year. We have the exclusivity for distribution the bee feed Ambrosia, made in Germany, we sell per year around 1.000 tons, in Spain, only in our group sell more than 500 tons per year of Ambrosia in autumn and Winter. Now we have agreements with companies from South Korea, to fight the news hornets in Spain and develop and adaptation the tecnolgy to the conditions in Spain. We travel every year to the mains fairs of beekeepers and honey buyers around the world, like Anuga in Köln, Sial in Paris, Gulfood, Sial China, Foodex Japan. Apimondia every edition, local fairs in Spain, Portugal, France, Germany and Italy. We export our production to countries, like Portugal, France, Germany, UK, Italy, Japan, Hungary, Morocco, Saudi Arabia, EAU, Canada and Mexico. We only sell products from our beekeepers.



### OP-133

## Atlas of Beekeeping in Brazil: a platform that gathers information about beekeeping to support decision-making

Ana Lúcia Assad<sup>1</sup>, Décio Luiz Gazzoni<sup>2</sup>, Heber Luiz Pereira<sup>3</sup>, Kátia Paula Aleixo<sup>1</sup>, Roberto Araujo<sup>4</sup>, Roberto Carvalho<sup>1</sup>

<sup>1</sup>Associação Brasileira de Estudos das Abelhas (A.B.E.L.H.A.)

<sup>2</sup>Embrapa Soja

<sup>3</sup>HP Agroconsultoria

<sup>4</sup>CropLife Brasil

Beekeeping has been a source of income for hundreds of thousands of people across Brazil. Besides being a strategic option to achieve sustainable development, beekeeping contributes to the pollination ecosystem services, assuring or improving crop yields and helps to maintain plant biodiversity in natural ecosystems. Aware of the need to better understand beekeeping in Brazil, the current challenges and opportunities, and the importance of its integration with agriculture, A.B.E.L.H.A. developed the Atlas of Beekeeping in Brazil (<https://abelha.org.br/atlas-da-apicultura-no-brasil/>). The platform integrates information about Brazil's beekeeping value chain available in different databases, including the Brazilian Institute of Geography and Statistics (IBGE, the acronym in Portuguese), the Brazilian website for free access to foreign trade statistics (COMEX STAT) and the Food and Agriculture Organization of the United Nations (FAO). The available data deal with the annual production and honey production value in the Brazilian states, as well as the number of establishments with beekeeping and hives throughout the territory. The Atlas also shows the distribution of crops (soybean, coffee, orange, lemon, melon, and watermelon) and forestry species (acácia mangium, acácia negra, algarobeira, bracatinga, and eucalyptus) that are of interest for agricultural pollination and to produce honey and other bee products. The data are aggregated on the platform as graphs, tables, and thematic maps. The platform is free to use for all those who need information or need to study this productive sector, such as public servants, researchers and students, in addition to beekeepers and farmers. By integrating data from various sources, the Atlas helps to improve comprehension of beekeeping in Brazil to qualify decision-making that leads to the professionalization and sustainable development of the activity in the country.

### OP-134

## Value Added Bee Product Training Program – The Fiji Experience

Karen Mills<sup>1</sup>, Karen Mills<sup>2</sup>, Anneliese Austin<sup>3</sup>, Anneliese Austin<sup>4</sup>

<sup>1</sup>Fiji Beekeepers Association

<sup>2</sup>Mudrenicagi Estate

<sup>3</sup>Bees for Sustainable Livelihoods

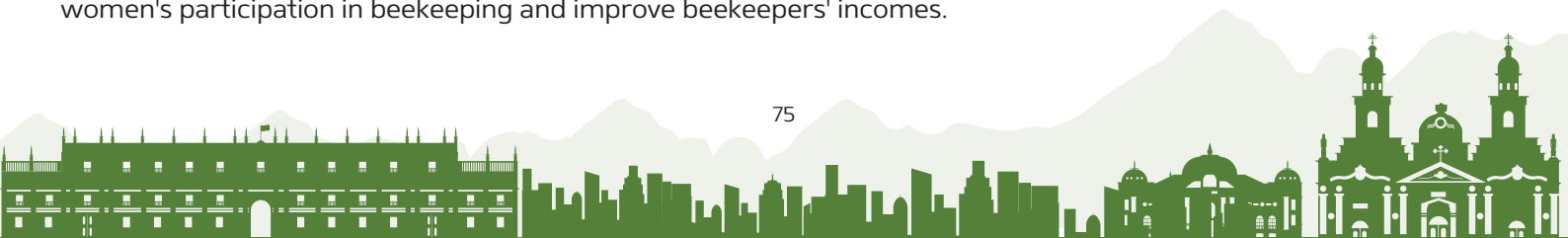
<sup>4</sup>Southern Cross University

During the past 30 years, Fiji's honey production has increased so that Fiji is now self-sufficient in honey production. However, sales of bee products have largely been limited to liquid honey, with many beekeepers discarding beeswax and having almost no awareness of other potential bee products. Additionally, beekeeping has been a male-dominated industry, while many women were seeking ways to contribute to household income.

In 2021, Fiji Beekeepers Association and Australian Centre for International Agricultural Research designed a program to promote production of value-added bee products to expand the market for bee products and to increase women's participation in the industry. The program included workshops on the production and sale of value-added products made from beeswax and honey, webinars and one-on-one mentoring for beekeepers on branding and marketing.

During the first two years of the program, 14 value-added bee product workshops were held for 123 students, 80% of whom were women. Results from post training surveys indicate a substantial positive impact on the number and value of value-added bee products produced and sold.

Promoting the production of value-added bee products has increased income for Fiji's beekeepers and created jobs for women in the industry. This program design can be adapted to other developing countries that seek to increase women's participation in beekeeping and improve beekeepers' incomes.



### OP-135

#### Insight into honey purchase and consumption processes in an emerging market

Rodrigo Romo<sup>1</sup>, Francisca Romo<sup>2</sup>, Carolina Correa<sup>2</sup>

<sup>1</sup>University of Bío-Bío

<sup>2</sup>University of Concepción

**PURPOSE:** Focusing on the Chilean honey market, this study aimed to analyze the determinants of honey purchase and consumption, and assess their impact on each of these two processes.

**Design/methodology/approach:** A questionnaire was specifically designed for this purpose and administered to a representative sample of 400 individuals, stratified by sex, age, and area of residence in the city of Chillán, Ñuble Region, which is one of the most important in honey production. Once the data were collected, a factorial analysis and two multinomial logit models were specified to analyze purchase and consumption.

**FINDINGS:** Unlike other agri-food products, honey is a product that does not have an expiration date, so the process of purchasing and consuming honey does not necessarily take place simultaneously. In contrast to the extant literature, this study suggests that honey purchase and consumption should be analyzed separately because they are affected by different variables.

**Originality:** The present study contributes to the state of the art on honey in an emerging market by collecting data on these processes and analyzing them separately.

### OP-136

#### A trade-off of honey bee breeding and beekeeping financial success

Borce Pavlov<sup>1</sup>, Sreten Andonov<sup>2</sup>, Bjorn Dahle<sup>3</sup>, Egoitz Galarza<sup>4</sup>, Marin Kovačić<sup>5</sup>, Janez Presern<sup>6</sup>, Aleksandar Uzunov<sup>7</sup>, Lazo Dimitrov<sup>8</sup>

<sup>1</sup>Company for Applied Research and Permanent Education in Agriculture, Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Skopje, Macedonia

<sup>2</sup>Swedish University of Agricultural Sciences, Department of animal breeding and genetics, Ulls vag 26, 75007 Uppsala, Sweden

<sup>3</sup>Norwegian Beekeepers Association, Kløfta, Norway

<sup>4</sup>ERBEL - erle beltz hazleen elkarte, Olaeta auzoa 6, Goizane eraikina, Zaldibia, 20247, Gipuzkoa, Spain

<sup>5</sup>Faculty of Agrobiotechnical Sciences Osijek, University of Osijek, Croatia

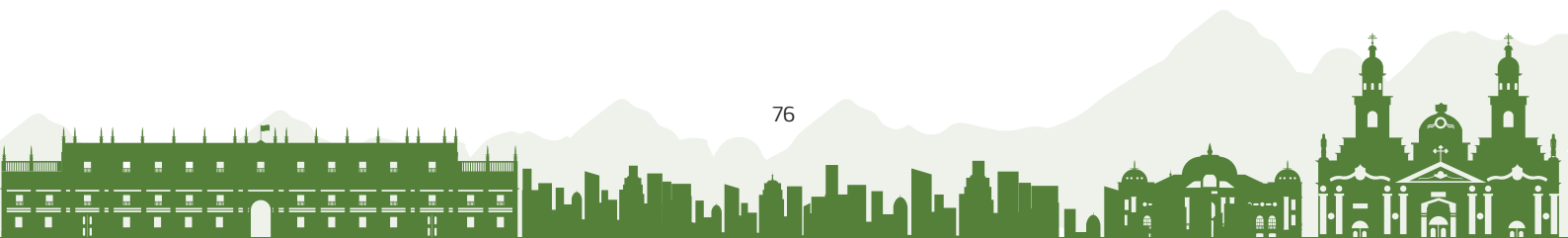
<sup>6</sup>Agricultural Institute of Slovenia, Hacquetova ulica 17, Ljubljana, Slovenia

<sup>7</sup>Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Skopje, Macedonia

<sup>8</sup>University Ss. Cyril and Methodius in Skopje, Institute of Agriculture, Skopje, Republic of Macedonia

Mating control in honey bee breeding is crucial to achieve selection progress. However, implementing it seems to be a major logistical and financial challenge for the honey bee breeders. In the framework of the BeeConSel project, we interviewed a total of 20 honey bee breeders from Croatia (HR, N=4), Macedonia (MK, N=6), Slovenia (SI, N=8) and Spain (ES, N=2) to collect data on economic aspect of queen production and mating. The honey bee breeders practised different mating control modes: geographical, biological (saturation) and temporal isolation or instrumental insemination. The analysis, revealed an average cost of production of one mated queen of 42.05 EUR, whereas the average selling price of a mated queen was 17.96 EUR, which results with negative difference of -24.09 EUR between the selling and the production price. Considering additional and higher costs due to controlled mating, the production price of a queen raises to 53.86 EUR and results with the highest negative difference of -34,15 EUR in componse with selling prices of controlled mated queens, while at the same time open mating queens have the lowest negative difference between the selling and production price (-11,79 EUR). Nevertheless, the analysis did not consider the positive economic implications of achieved selection progress due to applied mating control.

Breeders are aware of the importance of breeding locally adapted honey bees and of the fact that controlled mating of queens is one of the major aspects of achieving genetic improvement of stock. Less than 0.1 % of the marketed queens from HR, MK and SI mate under mating-controlled conditions, probably influenced by the much higher costs of controlled mating. However, to practice controlled mating without significantly affecting the price of the produced queens, breeders need professional and financial assistance in initiating, organising and implementing this critical aspect of breeding.





## Beekeeping Economy

### OP-137

## Decentralized Honey: Building Trust and Transparency in the Beekeeping Industry

Milan Wiercx van Rhijn

Facing Bees Foundation, The Netherlands

Honey adulteration is a major problem in the beekeeping industry, threatening the livelihoods of beekeepers and the viability of their operations worldwide. Chemical analysis, the current method of honey traceability, has reached its limits. While positive detection does not necessarily indicate honey fraud, buyers are reluctant to request additional analyses and may refuse further samples, reducing the chances for new honey providers to find markets.

Centralized solutions like permissioned traceability systems, audits, and certifications are expensive, require expertise, and demand a well-established infrastructure, excluding many smaller sub-industrial beekeepers and beekeepers in less developed areas and countries. Moreover, such centralized solutions lack transparency, audibility, and accountability.

To address these issues, an inclusive, low-barrier, and low-cost traceability solution that is accessible to any beekeeper is needed. This solution should be decentralized, enabling beekeepers to provide their own data, and allowing third parties to integrate with the system for decentralized, immutable logging.

The proposed solution, HoneyTrail, aims to provide such a solution. HoneyTrail sees chemical analysis as just one of the many factors of honey authenticity, allowing others to participate in trade that would otherwise be left out. Through this holistic approach, it will be harder for honey adulterators to cheat the system. Automated labeling of countries of origin can be achieved by immutably logging all the harvests, trades, and other actions.

In conclusion, a decentralized and inclusive solution is needed to combat honey adulteration, and HoneyTrail is one. The implementation of such a solution would not only protect the interests of beekeepers but also safeguard the environment and consumers while ensuring the availability of vital ecosystem services provided by bees.

### OP-138

## Key 2023 Issues About Honey Authentication

Federico Berron A.

FILAPI, IHEO, AMEMA

Who tests, what tests?

An update by Honey Authenticity Network, 1) Document IV with 3rd HAN analyses of retail honey jars from Mexico and UK, May 2023. 2) Past and future effects from contradiction of EA/LC-IRMS C3C4 analysis. Fraud industry benefited from analyses justifying 'no foreign sugars to honey' to samples of China syrups imports as honey into EU, UK, Asia & Africa and of India & Vietnam into US, millions of MT from 2008-2013.

What direction? A bright picture

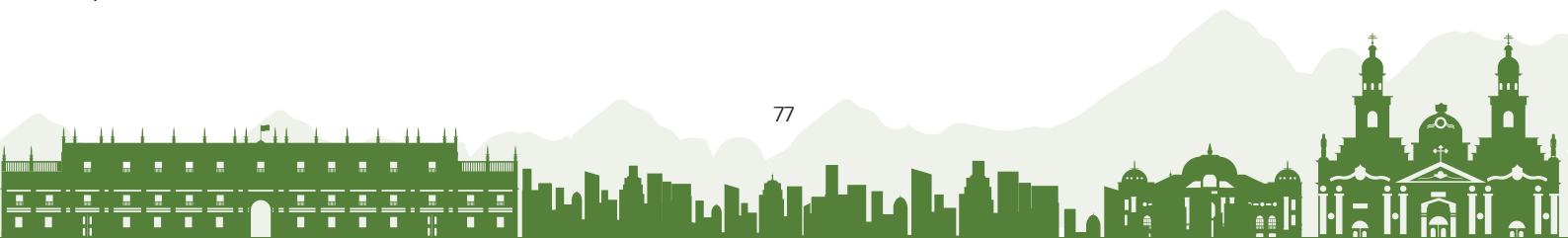
World Guild of Beekeepers is a HAN entity where expert beekeepers interact with Scientific Entities to protect honey as product of bees/beekeeper symbiosis, via open information and honey authentication systems based on the +180 components of honey and a continuously updated protocol. Honey is too rich in compounds to be replicated.

Of transparent origin

Simultaneously our database of honeys is accessible and traceable to each apiary, keeper & extraction dated size of production. Keepers do honey birth samples & register first transaction, further along the chain transactions and blends are sampled. year to year, enhancing the model until end consumer. This keeps terroir and honey identity and bees environment yearly changes.

Action

The transformation of the current fraud situation starts with two or three Consumers that ignite a Class Action and ask for an Injunction proposing Courts cautionary measures. Beekeepers affected in the UK & abroad can make a joint claim because it is all the same action and at some point in the supply chain the same defendant. Fraud amount has impacted above \$1 US Billion.



### OP-139

## Design and development of an effective communication strategy for the promotion of honey consumption in Chile

Antonio Sandoval<sup>1</sup>, Carol Acevedo<sup>2</sup>, Enrique Mejias<sup>3</sup>

<sup>1</sup>Abeille Consultores

<sup>2</sup>Federación Red Apícola Nacional F.G.

<sup>3</sup>Centro de Tecnologías Nucleares en Ecosistemas Vulnerables CTNEV. División de Investigaciones y Aplicaciones Nucleares DIAN. Comisión Chilena de Energía Nuclear CCHEN

In Chile, different types of honey are produced according to the geographical origin. As a result, it is possible to find honeys with a wide variety of organoleptic attributes, including a great variety of flavors, colors, and textures. Likewise, honey production in Chile is distributed throughout the country, although the largest production volumes are concentrated in the Regions of Maule, Biobío and La Araucanía.

Despite the recognized attributes of honeys, per capita consumption in Chile remains low and Chilean consumers do not have enough knowledge about the value of Chilean apiculture products. By the other hand, there are wrong interpretation and misunderstood data about fake products which increase the bad perception of consumers when they buy any given honey.

Promotion and dissemination have been done largely using social networks and short communications through instant messaging. Most of these efforts have been carried out as independently self-managed initiatives and have aimed to highlight the goodness of honey and the importance of bees, but they have not been effective enough to reverse the domestic consumption scenario in the country.

Until now, there has not been available a promotional campaign for Chilean honeys with a powerful centralized message and whose story allows to make visible the advantages of honey consumption using the prevailing communicational tools nowadays.

Here we present the design of an effective communication campaign for the promotion of Chilean honeys based on the strategy developed by Abeille Consultores in 2018 and 2019 for the annual celebration of the Honey Day in Chile and the appropriate adaptation to the current national post-pandemic situation.

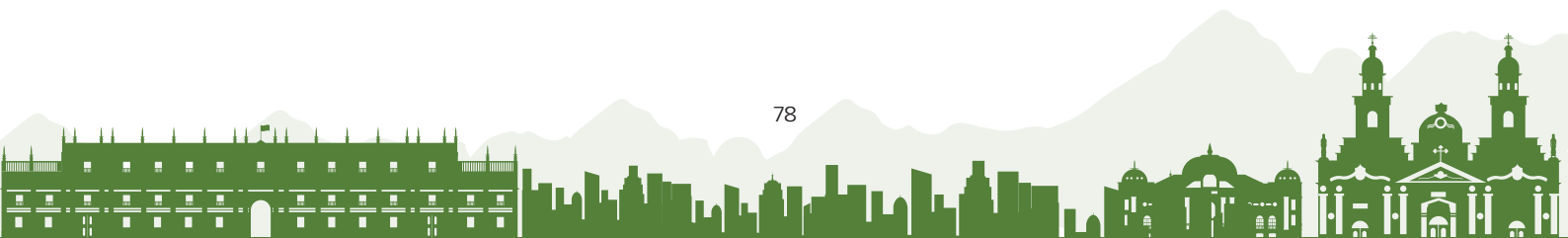
### PP-255

## Raising Resilient Bees in rural pristine watershed located in Oregon, USA using closed breeding program and naturally reared queens without the use of grafting in commercial apiary family-sized operation

Eric McEwen, Joy McEwen

Adaptive Bee Breeders Alliance, member

Authors, Eric and Joy McEwen, share insights from their new book, Raising Resilient Bees, about their journey as an organic beekeeping family operation and their unconventional means of developing an inbred line of honey bees with increased levels of resistance to varroa mites. This long-term selection program when paired with management practices that include integrated pest management techniques to reduce varroa mite populations has allowed minimal use of acaricides, specifically oxalic acid twice annually. Using in-hive queen propagation techniques in lieu of grafting, daughter colonies are raised annually from every extant colony. By maintaining relatively high mite pressure, we have imposed a selective force on our inbred line toward mite resistance. Differential rearing of number of daughter colonies per over-wintered colony allows for steady genetic transformation of our meta-population. By foregoing grafting, we prevent unintended loss of rare genetic information. We use a “soft bond” method of minimalistic intervention to maintain the population and preserve genetic diversity, by limiting genetic losses and prevent genetic bottlenecks. In eight years, our number of colonies has never gone below 250. Initial evidence suggests greater survivorship in colonies threatened by varroosis.



## Beekeeping for Rural Development

### OP-140

#### **A course concept for a beekeeping method in the rural area of Lower Saxony / Germany with low material input - basis for sustainable beekeeping with students, also with disabilities, enabling inclusive learning**

Ines Oldenburg

Department of Educational Sciences, Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany

The presentation introduces a course concept for non-professional beekeepers, which is implemented in the rural area of the Northwest in Lower Saxony / Germany. The special way of beekeeping with the Dadant system taught there allows sustainable beekeeping with smallest interventions on the bee colony. The use of materials for hives and tools is low. The course concept is documented with low barriers according to the principles of UDL (Universal Design for Learning), so that course participants with special impairments (disabilities) can also learn with it. The course concept is used by teachers in the region to set up a school apiary at their schools and thus give students a practical understanding of the principles and interrelationships of selected areas of the 17 Sustainable Development Goals (SDGs) using the example of beekeeping and honey harvesting. Appropriate teaching materials were also developed for this purpose, enabling joint, inclusive learning by students with and without disabilities.

The presentation introduces the course concept and the teaching materials and shows examples from the implemented practice at a school in Lower Saxony.

### OP-141

#### **Breaking the Cycle of Poverty by Beekeeping with Indigenous Bees – Field Studies from India**

Dhanshree Chavan

Donor Engagement, Under The Mango Tree Society, Mumbai, India

About half of India's population still relies on agriculture with 86% farmers being small and marginal farmers. These smallholders play an important role in food security and face a critical challenge of decline in productivity due to limited access to agricultural inputs and lack of information, resulting in low incomes, no savings and limited chances of reinvestment in agriculture.

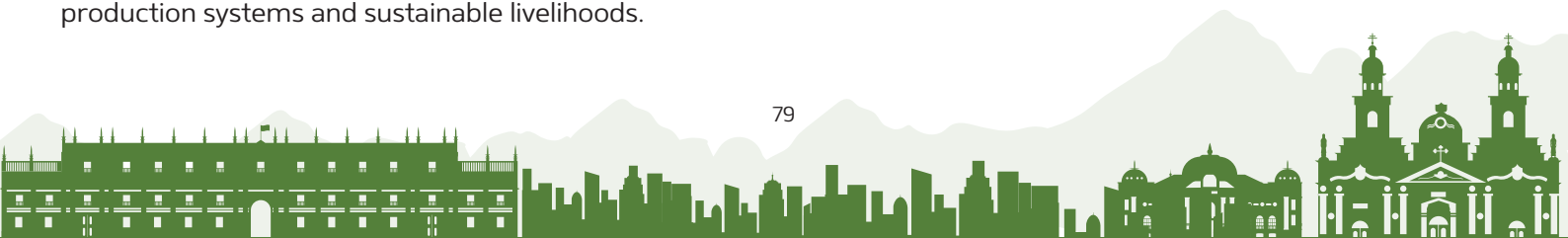
Under The Mango Tree Society's (UTMTS) Bees for Poverty Reduction Programme plugs this gap of information and skill building by helping smallholders increase their yields with beekeeping. Selected farmers are trained in beekeeping and are provided with continuous handholding support at farmgate to help them learn the nuances of beekeeping.

Beekeeping with the local indigenous bee species is well-suited to the diversified farming systems found in smallholder communities in India. Input costs are low because the bees are locally available, resilient and are well adapted to the local environment, they subsist on existing flora and are excellent pollinators.

Farmers associated with UTMTS have experienced pollination impact on vegetables (flat beans and gourds, 20%-25%), agricultural (pigeon pea and black gram, 20%-30%) & horticultural (mango and cashew, 15%) crops and have been able to harvest clean honey and wax sustainably, with the only investment being time for training and willingness to upskill. The presence of bees has also led to reduction in expenses of buying chemical pesticides resulting in improved savings to be re-invested in agriculture.

The beekeeping ecosystem developed by UTMTS, also facilitates beekeepers to purchase beekeeping equipment locally at reasonable rates if they wish to scale-up income around beekeeping. This arrangement creates local livelihood opportunities and improves access to cash-in-hand by increasing income avenues. Many innovations around the beekeeping value chain like addition of kitchen gardens and bee friendly flora have further enhanced income for smallholders.

The presentation will share data from primary surveys on yield and income impact from beekeeping from UTMT Society's field areas alongwith some case studies. These show that beekeeping with indigenous bees can break the circle of extreme challenges brought by poverty, ignorance and climate change and lead communities to viable production systems and sustainable livelihoods.



## Beekeeping for Rural Development

### OP-142

### Some Tried and Tested Ways to Promote Beekeeping among Rural Youth, especially Women – Field Experiences from India

Dhanshree Narayanrao Chavan

Donor Engagement, Under The Mango Tree Society, Mumbai, India

To combat the issue of development in both farm and non-farm sector especially in countries like India where agriculture is the mainstay of the rural economy, skilling rural youth is of utmost importance.

Under The Mango Tree Society has pioneered an innovative Bees for Poverty Reduction programme where close to 11,700 beekeepers have been trained over 14 years. Some of the key features of the programme are that training happens over the course of the beekeeping cycle (typically a year), at the farmgate. A local cadre of specialized Master Trainers are also developed alongwith so handholding support can be extended when needed.

The way these training modules are designed ensures that long term sustainability is achieved and results in self-sufficiency of the participants continuing beekeeping. Bee flora and kitchen gardens developed using low cost methods also form an integral part of the programme resulting in improved engagement of participants especially women.

Some of the strategies used to ensure that rural women attend training and continue to remain engaged to transition into successful beekeepers are - locally organized training at a venue accessible to women on mutually agreed days, delivering content in dialects they understand, preferably all women groups, continued support post-training, addressing challenges faced and help network internally.

Over the years, it has been observed that engaging young women from rural communities has improved nutritional intake of families, increased savings and increase in income from sale of small surpluses generated due to pollination impact post-beekeeping. Some of the trained women beekeepers have graduated to Master Trainers and technical experts with the motto of Each to Teach and Reach, inspiring others to take-up beekeeping with indigenous bees. Most of these women share same stories either- they were scared of bees initially and are now comfortable handling bees or they themselves practiced traditional honey hunting but were never approached for trainings due to gender biases.

### OP-143

### Tools for strengthening productive capacities in beekeepers from the Central Pacific Region of Costa Rica

Paola Hernández-Ching, Sánchez Luis

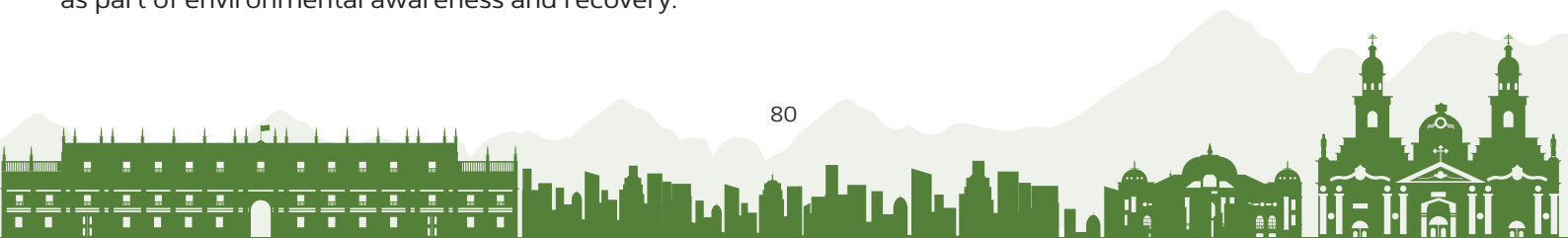
Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, Heredia, Costa Rica

By implementing this project, the aim was to promote the empowerment and productive improvement of beekeepers operating in an area of high ecological degradation and marked poverty levels in the Central Pacific Region of Costa Rica, for whom beekeeping is their main source of income. Based on these criteria, more than 30 productive units have benefited by obtaining tools and knowledge to optimize their productive and commercial practices, which can result in improving their quality of life and growth expectations, while mitigating the processes of poverty and unemployment accentuated by the COVID-19 pandemic.

In addition, the participation of actors representing the triple helix model of innovation was incorporated: government (municipalities and Ministry of Agriculture and Livestock), academia (CINAT-UNA), and the productive sector (National Chamber for the Promotion of Beekeeping, sponsors: UNDP and GEF). A work methodology was implemented in stages, where the productive profile of the participating individuals, their main problems, and their needs were obtained through the application of semi-structured interviews. Based on this information, core activities were designed, and the content of talks and workshops according to four thematic nuclei:

1. Best practices in beekeeping production
2. Administrative management of beekeeping companies
3. Value-added generation with bee colony derivatives
4. Education and environment

As a tangible result, 26 training and workshops were conducted with more than 140 people, 6 learning guides were developed in areas of business management diversification with bee colony derivatives (confectionery, cosmetics, and artisanal beer with honey), and a teaching support guide for working with bees. Labels for 7 brands of honey from small producers were also designed, and support was provided to beekeeping companies for the creation of business accounts on social media. An innovative aspect was the development of an educational app for mobile devices, as well as a teaching support guide for learning about bees, and the planting of 750 honey-producing trees was encouraged as part of environmental awareness and recovery.



## Beekeeping for Rural Development

### OP-144 Utilizing Indigenous Beekeeping Knowledge

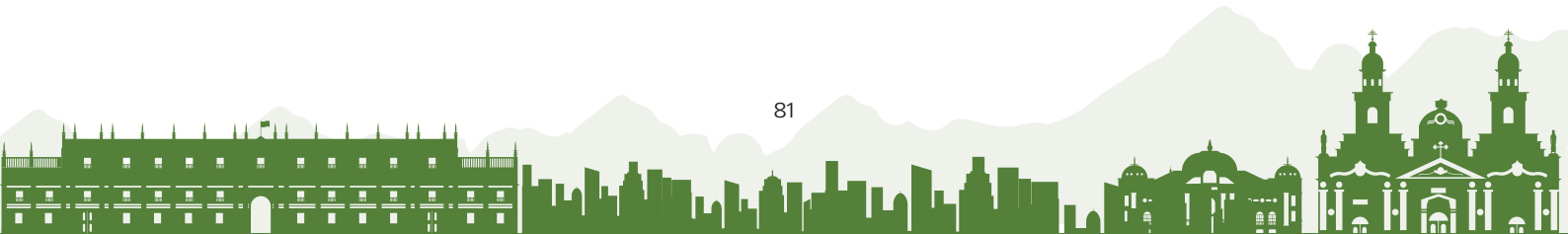
Robert Nil Mutisi  
Working for Bees Private Limited

The issue of indigenous knowledge systems in beekeeping and adaptation strategies to poverty, hunger and climate change has gained serious attention to researchers. The indigenous knowledge systems (IKS) have opened serious debate with contrasting views within society. Some people argued that indigenous knowledge systems have got space in beekeeping for rural development while some arguing that they are associated with several drawbacks. Unfortunately, little has been done in Zimbabwe to document indigenous knowledge systems hence the purpose of the study. The study was undertaken to identify, record and document those IKS of beekeepers that could be used to make beekeeping for rural development sustainable, affordable, viable and nature-based. Decisions during planning, designing beekeeping and tree flowering calendars, as well as informing preparedness against disasters that affect bees and other pollinators would be based on the documented information. In this study, the writer interrogated indigenous knowledge systems' roles in beekeeping focusing on seasonal prediction, linguistics, craft skills, hive styles, hive construction and source of material, apiary management, botany, security, medicine, swarm catching, honeybee pests and diseases, colony control and protection methods, dealing with stings, women and youths participation. Three districts of Manicaland province participated in the study with 20 participants purposively chosen from each district. Data were collected using semi-structured interviews, focus group discussions, key informants interviews, and field observations. The findings of the study revealed that IKS play a critical role in beekeeping for rural development. The results showed several characteristics of indigenous knowledge systems that strengthened the importance of IKS in beekeeping for rural development. Results from the focus group discussions brought Christianity, Western education and climate change as major threats to indigenous knowledge systems. The reliability of IKS is being challenged and reduced by the given factors and its susceptibility for disappearance was found to be increasing. The study concluded that IKS be included in policy frameworks and in the planning phase of rural development programs. Lastly government and development partners were recommended to develop policies and procedures that accommodate indigenous knowledge systems in rural development initiatives.

### OP-145 Exploring the Benefits, Barriers, and Opportunities of the USAID Farmer-to-Farmer Honey Bee Program in the Dominican Republic

Amy Vu<sup>1</sup>, Maria Montas<sup>2</sup>, Francisco Mendez<sup>2</sup>  
<sup>1</sup>University of Florida  
<sup>2</sup>USAID Farmer to Farmer Program, Dominican Republic

**BACKGROUND:** The USAID Farmer-to-Farmer Program provides technical assistance in developing countries to farmers and others in agriculture to promote best practices that help increase business profitability and strengthen agriculture. The results of programs are sometimes unknown and there may be some challenges that participants, field officers, and volunteers face. **OBJECTIVE:** One objective of the University of Florida (UF), Institute of Food and Agricultural Resources (IFAS), Honey Bee Research and Extension Laboratory (HBREL) is to offer valuable beekeeping education to beekeepers and beekeeping educators. The University of Florida was involved in a Farmer-to-Farmer program in Jarabacoa, Dominican Republic, training backyard and sideline commercial beekeepers focused on best management practices and queen rearing techniques in February 2023. The study focused on exploring the benefits, barriers, and opportunities of this specific program in the Dominican Republic and the overall challenges with USAID Farmer-to-Farmer programs. **METHODS:** The program was a 15-day training with a cohort of beginner and established beekeepers. Beekeepers had been keeping bees for less than three years. None of the participants were full time beekeepers. The program involved site visits and hands-on classes in the apiary. In the program, there were two volunteers and two field officers. Video interviews were conducted on the last day of the program with participants and two field officers. **RESULT:** 13 participants attended the program each day, and 10 were video interviewed. The participants provided significant feedback regarding what they learned in the program. Specifically, they mentioned lack of resources and minimal funds preventing them from keeping bees efficiently. 100% found the training a good use of their time and felt motivated to continue their education and participation in the local beekeepers association. **CONCLUSION:** There are many challenges, yet opportunities, for those who participate in the Farmer-to-Farmer program as a volunteer, participant, or field officer. International collaboration between Extension specialists, beekeepers, government entities, and apiary stakeholders can lead to successful programming in apiculture worldwide.





## Beekeeping for Rural Development

### OP-146

#### Intervention of modern movable frames in the traditional wall hives for sustainable ecosystem of Garhwal division, Uttarakhand, India

Atar Singh Kaintura

Shivalik Natural Products

Indigenous honey bee *Apis cerana* beehives are a part of the architecture of mountain regions of Garhwal, India. Wild colonies of *A. cerana* used to inhabit in wall hives known as Khadra. A Wall hive is a cavity left in the wall when the house is under construction; usually located at the height of about 150 cm from the floor. Wall hives are more suitable for temperature control in heavy rainfall/snowfall areas and the hives are also protected from wild animals and predators. Honeybees not only provide honey but also help in pollination of flowers to continue the progeny of plants. Thus placement of hives in an area had an important role for the sustainable ecosystem. Even though there is vast potential of flora for beekeeping in Garhwal region, the bee population is declining due to destroy of the combs while honey harvesting. Decreasing population of *A. cerana* tends toward the loss of our social and cultural heritage. During current study efforts were made to design low cost, eco-friendly movable frames. Provision was made with wooden frames in Khadras. Natural fixed combs prepared by the bees are replaced to the movable frame. Inside the wall, frames are vertically hung parallel to each other. Extra frames are filled with sheets of wax foundation on which the combs are built by the bees. Scientific intervention in the indigenous method makes convenient for inspection of the colonies, pest management, swarm control and division of bee colonies. Honey is harvested hygienically in honey extractor, thus facilitates to get repeated honey harvesting in the season and thus increases rural economy due to high quality and quantity of organic honey production. Awareness was created and training is provided to the villagers to propagate *A. cerana* bee colonies, through the use of movable frames in wall hives. Thus improving of the bee population is possible for sustainable ecosystem.

### OP-147

#### Challenges in the implementation of Revolving Funds in beekeeping. The case of the Cámara de Apicultores Pampero (Argentina)

María Emilia Estrada<sup>1</sup>, Elian Tourn<sup>2</sup>, Gabriel Gómez<sup>3</sup>, Diego Iaconis<sup>4</sup>

<sup>1</sup>Departamento de Economía, Universidad Nacional del Sur; Laboratorio de Estudios Apícolas (LabEA - UNS CIC)

<sup>2</sup>Departamento de Agronomía, Universidad Nacional del Sur; Laboratorio de Estudios Apícolas (LabEA - UNS CIC), Cooperativa de Trabajo Apícola Pampero Ltda.

<sup>3</sup>Cooperativa de Trabajo Apícola Pampero Ltda.

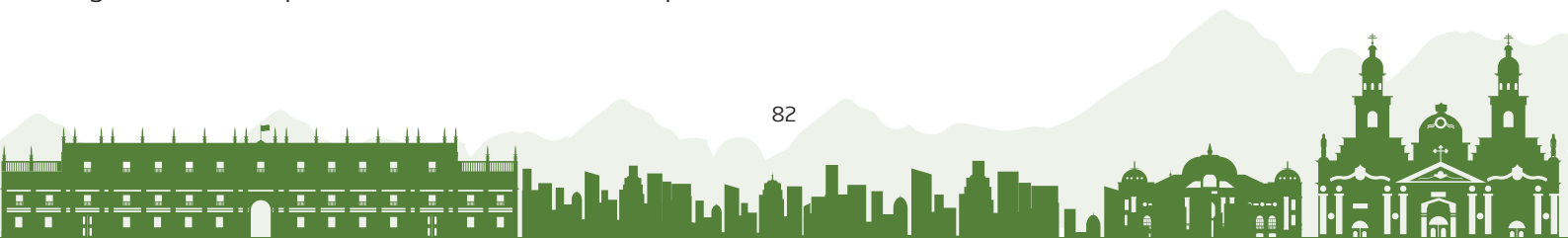
<sup>4</sup>Cámara de Apicultores Pampero

One of the most significant constraints to the development and growth of small and medium-sized beekeepers is access to the financial resources offered by the banking system. Banking requirements act as a barrier to access for these credit subjects considered very risky, given their income structure, the lack of the type of collateral required and their level of capitalization. Meanwhile, other financing options are significantly more expensive. In this context, Revolving Funds (RF) are an alternative to bank financial exclusion.

The RFs implemented from associative strategies, with primary financing generally subsidized by the State, Non-Governmental Organizations (NGOs) or international cooperation agencies combined with the beneficiary organizations' own resources, circulate or rotate between the organization and the producers. The objective of the beneficiary organizations is to sustain or increase these funds in the long term to multiply the possibilities of credit in order to promote productive and social transformations. To this end, they establish a menu of mechanisms that allow for responsible management of resources (such as the involvement of members in decision-making, solidarity groups as guarantors) and a high rate of collectability, and consider the particularities of production, without discouraging the taking of credit.

Likewise, the criteria established for taking loans demand periodic revisions, either due to aspects not previously considered, or due to the sectoral context and/or macroeconomic issues. These adjustments and modifications must be agreed upon without weakening the organization's spirit of solidarity.

This work sought to show the lessons learned when implementing and self-managing FR within the framework of associative strategies that bring together beekeepers. As a case study, the paper analyzes the implementation of Revolving Funds by the Cámara de Apicultores Pampero (Coronel de Marina Leonardo Rosales, Buenos Aires, Argentina) and its performance from 2021 to the present.



## Beekeeping for Rural Development

### OP-148

#### Socio-environmental impact of the production and commercialization of honey in the buffer zone of the Gorongosa National Park

Marcos Bera Chova<sup>1</sup>, Aurelio De Jesus Rodrigues Pais<sup>2</sup>

<sup>1</sup>Beekeeping Supervisor, Gorongosa National Park

<sup>2</sup>Center for Ecology and Natural Resources Studies, Zambeze University, Mozambique

The production and marketing of honey in the buffer zone of the Gorongosa National Park (GNP) has resulted in a positive socio-environmental impact on the quality of life of local communities. This region has been cyclically affected by drought and floods in recent years, associated with climate change, which results in losses of agricultural areas. Honey has been one of the non-timber forest products extracted and marketed as an alternative for survival, as well as for the treatment of respiratory problems and wounds caused by burns. The promotion of the honey value chain in the buffer zone by the PNG program has contributed to changing the financial conditions of communities and conserving biodiversity. The present study was conducted using a questionnaire containing open and closed questions to 400 households in the PNG buffer zone, and it was found that 54% produce and sell honey. It also made it possible to know some forest species where bees collect nectar and pollen, such as *Acacia robusta*, *Combretum microphyllum*, *Julbernardia globiflora*, *Kigelia africana*, *Philenoptera violacea*, *Ziziphus mauritiana*, *Lecaniodiscus fraxinifolius*, *Ozoroa obovata* and *Ziziphus mucronata*. Due to the benefits of honey production, today it appears that forest fires are controlled and avoided by local communities as a way to prevent the abandonment of bees in hives and the decline in production. The commercialization of honey has increased family income, thus allowing the acquisition of school material for children and the purchase of food, since the first honey harvest season occurs at a time when agricultural products are not yet ready for consumption, from January to April.

### OP-149

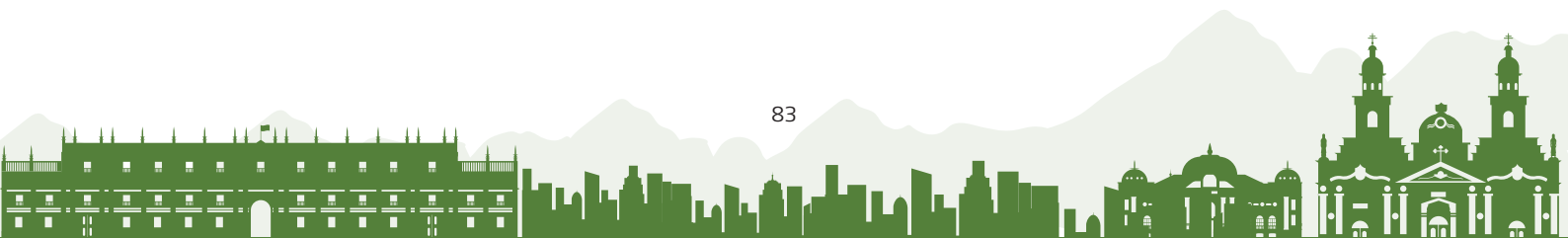
#### Beekeeping as an alternative economic in Anorí, Antioquia-Colombia

Sandra Milena Diaz Puentes<sup>1</sup>, Oscar David Rojas Hernández<sup>2</sup>

<sup>1</sup>Stadual University of Maringa, National University of Colombia

<sup>2</sup>National University of Colombia

Anorí, located in the department of Antioquia-Colombia, is part of the municipalities with Development Programs with a Territorial Focus. These programs are developed in territories most affected by violence, poverty, etc. and that seeks through viable alternatives to contribute to rural development. Within these, beekeeping has been implemented to benefit 25 families, with the aim of helping the family economy, avoiding entry into illegal activities. In this work, personalized visits were made to each beekeeper (25), where socioeconomic information was collected. It was identified that 68% have been victims of the armed conflict, either due to forced displacement or loss of family members by illegal armed groups; 16% are under 40 years old, 52% between 40-60, 28% between 60-70, demonstrating a low participation of the young population. At the educational level, 24% have incomplete basic education, only 8% completed their secondary education, 20% are technicians and 8% professionals, reflecting on their income level in which 32% receive less than a USD \$224 and 20% more than USD\$ 224. Regarding gender, 64% are men. Beekeeping began less than seven years ago in Anorí, through a project that gave the colonies of Africanized honeybee without previous training to the families and without continuous and good quality technical assistance. Due to the above, it is still a more traditional activity, on average each apiary consists of 9 colonies, and the average honey per colony is 7 kg/year, however, the kilo can be sold for USD \$13.5. Because of this, it is a sideline economic. Being more beneficial for women, housewives, because with that income they have some economic independence and limit illegal activities for their livelihood, also serving as protection for the forests of their properties. The above, evidence that beekeeping can be a means of rural development, its main difficulty is the lack of technical assistance that leads to low productions, but with adequate support, it could become a sustainable economic primary activity.



## Beekeeping for Rural Development

### OP-150

#### Analysis of Production and Marketing of Beehive Crops in Adamawa State, Nigeria

Muhammad R. Ja'afar-Furo, Yahya A. Dauda

Department of Agricultural Economics and Extension, Faculty of Agriculture, Adamawa State University, PMB 25, Mubi, Nigeria.

The study assessed the production trend and marketing of beehive crops in Adamawa State, Nigeria. A total of 120 apiarists were selected through a snowball sampling method. Data were collected by the use of structured questionnaire and supplemented by group discussion, and analysed by application of descriptive statistics, Net Farm Income (NFI) analysis and multiple regression. Results showed that all (100%) the beekeepers were male within the middle aged group, mostly married and earning income between N50, 000 – N100, 000 per annum. Majority of the apiarists used traditional beehives, had household size of 5-10 persons with a larger proportion fairly enlightened and most of them having experience of more than 20 years in beekeeping. The most influencing factors of production of the enterprise in the area were number of beehives in the apiaries, levels of experience and education of apiarists ( $p < 0.001$ ). Apiculture was found to be highly profitable amongst the beekeepers in the State, with a very efficient marketing system. Conclusively, it could be stated that beekeeping was a male dominated enterprise in the study area and improved skills of enterprise management was discovered to be highly significant in determining yield. It's highly recommended that policymakers should introduce low-technology beekeeping in the area for the purpose of reducing poverty and serve as avenue for creation of employment among the rural communities.

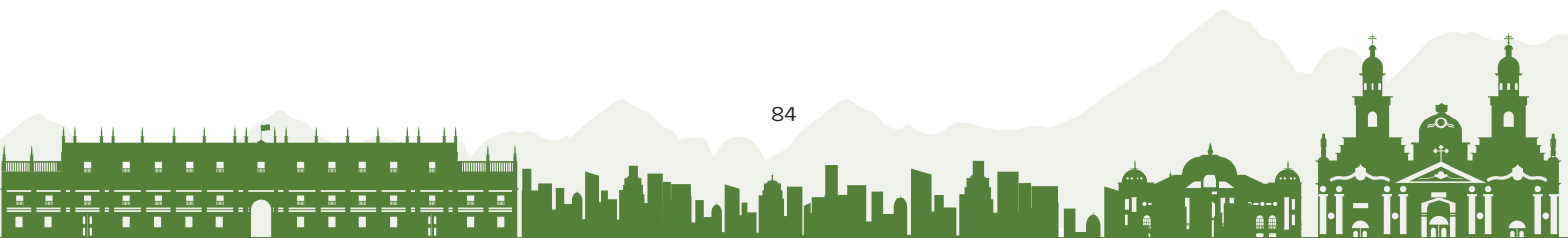
### OP-151

#### Multiplication Of Honeybee (*Apis Mellifera Adansonii* L.) Colonies Using Three Different Low-cost Model Nucleus Hives Design In Two Main Vegetation Zones Of Nigeria

Akande Alabi Ayoade, Oyerinde Abolade Akeem

Department of Crop Protection, University of Abuja, Abuja, Nigeria

In Nigeria, there is a growing need for additional honeybee colonies to satisfy the demand for pollination services and compensate for high colony mortality through bush burning, vandalization and pesticide poisoning. The main objective of this study was to multiply honeybee colonies using different models of nucleus hives. This research is poised to build beekeepers capacity in the use of "Waste to Wealth" forms of nucleus beehives to maximize production of hive products and pollination services. The study evaluates the preference of different nucleus hive to the establishment of colony and how to mitigate the effect of pest and diseases in the apiary. This was carried out in the two main agroecological zones of Nigeria (i.e., forest and savanna). The study sites were the Teaching and Research Farms of Faculty of Agriculture, University of Abuja (8 °58'N, 7 °10'E) and Obafemi Awolowo University (7° 31'N, 4° 31'E) in the savanna and forest vegetation respectively. The data collected includes the rate of colony establishment, number of incoming bees, colony weight, brood length, incidence of pest infestation and infection of diseases as well as colony abscondment rate at week intervals. The experiment at the two locations was laid out using Randomized Complete Block Design (RCBD) and data collected were analysed using SPSS Version 22. A total number of 24 nucleus hives made from different low-cost model of materials were deployed in the two agroecological zones. The result shows that the bamboo and the carton hives ("Waste to Wealth" hive type) gave similar result with the conventional wooden hives. The findings from this study portrayed that bee farmers in Nigeria can adopt the use of low-cost materials of bamboo and carton to raise nucleus hives for establishment of colony in modern beekeeping practice. This will help the commercial bee farmers to increase their colony in a short time without the use of wooden hive and in turn lower pressure on the demand for planks for building hives and in the shortest time reduce deforestation and assures a healthy global environment.



## Beekeeping for Rural Development

### OP-152

#### Women beekeeping during the war in Ukraine

Leonora Adamchuk<sup>1</sup>, Dina Lisohurska<sup>2</sup>, Iryna Kovalchuk<sup>3</sup>

<sup>1</sup>National University of Life and Environmental Sciences of Ukraine, National Research Center «P.I. Prokopovich Institute of Beekeeping», Public Organization «Foundation of Women Beekeepers», Kyiv, Ukraine

<sup>2</sup>Polissia National University, Public Organization «Foundation of Women Beekeepers», Zhytomyr, Ukraine

<sup>3</sup>Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Public Organization «Foundation of Women Beekeepers», Lviv, Ukraine

The NGO Foundation of Women Beekeepers is a non-profit organization in Ukraine that promotes the importance of bees for food security and introduces beekeeping practices while protecting bees. The organization focuses on developing female leadership in beekeeping and supports women in villages to help them develop their own businesses. They conduct vital strategic research and educational initiatives that protect bees and promote the introduction of managed bee pollination and increase food security. In 2022, the quality standard COU 74.9-43300769-001: 2022 was adopted, standardizing the spheres of activity of the NGO "Foundation of Women Beekeepers." According to the standard, the interests of the organization above one's own interests, has a policy of non-discrimination and promotes the development of a powerful, effective, and mass feminist movement. The organization's vision is that bees and women can save the world with their beauty, harmony, hard work, and wisdom. The mission is to develop together, preserving the identity of each. The purpose of the work is to analyze the impact of the Russian-Ukrainian war on the activities of the Organization. The organization had 60 members from 18 regions of Ukraine from 2019 to 2021, but due to bankruptcy, change of activity, travel abroad, and other reasons, the number of members has decreased to 40 by May 1, 2023. In 2022, their main activities included providing direct assistance against the enemy, charitable and volunteer activities for the military, migrants, and victims, representation in the Working Group under the Ministry of Agrarian Policy and Food of Ukraine, solving strategic issues of conservation and post-war restoration of beekeeping, representation at the international level, development of the Foundation and its members, and professional activities of members. Due to the economic crisis, limited logistics, risks of destruction of agriculture and home, and occupation, the organization faced challenges in carrying out its activities. Despite the challenges, the organization made several achievements in 2022-2023, such as the development of volunteer and charitable activities, quality standards, renewal and development of the brand, active educational and training work, and the development of bee pollination direction and preservation of pollinators.

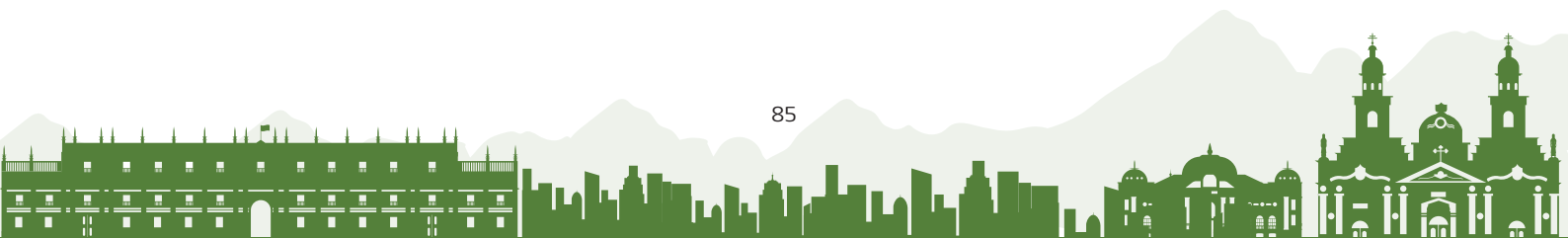
### OP-153

#### How I raise quality queen bees in a changing world

Tucka Saville

Tucka Bee LLC, Florida, United States

Amidst climate change, lack of resources, routine pesticide use and habitat loss, it can be difficult for beekeepers to find quality queens. This talk describes some small-scale solutions that have worked for me. I produce over 1000 queens each year from about 400 colonies in Florida and New York, United States. I make the boxes, raise most of the cells, negotiate with the landowners and source re-used materials locally at minimal cost. I maintain a wide net of connections with local beekeepers and farmers in my area. They keep tabs on the weather, sell my products, and plant large quantities of forage for the bees. The small size of my operation allows me to leave the mating nucs alone for 3-4 weeks after cell placement. The bees are very healthy because they get a consistent break in the brood cycle each time I sell a queen. My customers are pleased with the high quality of my product, and happy to pay a fair price. Each year, I train an intern or two to run a similar small operation in their own area. As my friends and colleagues also do this, I am very happy to be part of a growing, mutually supportive community of small scale queen producers across the United States.



## Beekeeping for Rural Development

### OP-154

#### Nature based beekeeping in a commercial and educational setting in rural south west England, UK

Paula Carnell

Paula Carnell Limited

Many land owners with visitor attractions and hotels, are wanting to keep bees, believing that is the best way to 'save and support bees'. Without a thorough initial audit of the native solitary and bumble bee species, introducing honey bees can be devastating for biodiversity. By working with land owners before they introduce bees, a sustainable bee plan can be established, setting up bait hives to capture wild native honey bees, and developing stories and awareness about the bumble and solitary bees already in the area beneficial for pollination.

Using nature based beekeeping and the native bee populations, setting up a variety of projects across the UK from Hotels and museums, wellness retreats and golf courses ensures increased biodiversity as well as educating landowners and their customers to the complex issues of bees and the environment.. A return on investment can be created from bee related products and experiences, not putting unnecessary pressure on bees in areas, such as the UK, where generous honey harvests are not guaranteed.

The first step is a thorough 'bee audit' of the area to calculate the number of bee species currently in the area. This includes solitary, bumble bees and honey bees. Locating wild colonies of honey bees is also vital in maintaining a balanced natural ecosystem.

Once an awareness of the local bee species is available, bait hives can be positioned to capture swarms from the local environment. These bees can then be managed in more conventional hives. One project in Somerset began with 2 wild colonies of honey bees, and 8 species of solitary and bumble bees. In six years, the numbers increased naturally to over 15 colonies of honey bees and 26 species of other native bees. All honey management was without any chemical treatments and no sugar feeding. This project alone inspired much public interest and is inspiring change in conventional intensive bee management.

### OP-155

#### Socio-economic analysis of the impact of beekeeping in in the rural livelihood of the Kaudwane community

Yari Roggia<sup>1</sup>, Melusi Rampart<sup>2</sup>, Alessandro Araldi<sup>3</sup>, Valentina Isaja<sup>1</sup>, Margaret Peggy Mutakela<sup>4</sup>, Monica Vercelli<sup>5</sup>

<sup>1</sup>Fondazione Zoom, Strada Piscina, 36, 10040, Cumiana, Turin, Italy

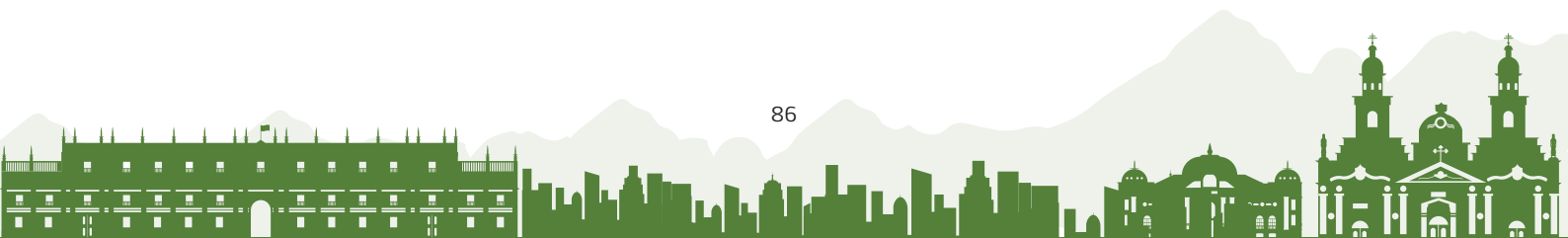
<sup>2</sup>Botswana University of Agriculture and Natural Resources, Private Bag 0027, Gaborone, Botswana

<sup>3</sup>Leopard Ecology & Conservation, Khutse G.R., Botswana

<sup>4</sup>Dept. of Animal Production, Private Bag 0032, Gaborone, Botswana

<sup>5</sup>Independent Researcher, Turin, Italy

Apiculture has transformed ecological and conservation research. However, the impact of bees and bee products on the socio-economic diversity in rural areas of Botswana has been neglected. The present collaborative research is between BUAN University (Botswana), Leopard Ecology & Conservation (Botswana) and Zoom Foundation (Italy). The study wants to investigate the socio-economic impact of beekeeping to the rural community of Kaudwane. The area is a typical semi-arid savanna with rich flora and proper ecological conditions for the existence of natural colonies. Although Botswana has achieved a high economic growth rate since independence, the country is still experiencing high levels of poverty especially in rural areas. With the majority of Botswana's poorest people living in uncertainties occasioned by changing environment, a variety of livelihood strategies such as beekeeping need to be adapted and improved to ensure sustainable livelihoods, especially in the fragile ecosystems. The beekeeping sector plays a major role in rural socio-economic development and environmental conservation. Beekeeping is a source of food (honey, pollen, royal jelly and brood), medicinal purposes (honey, propolis, and bee venom), tourism (apitourism, educational activities) and raw material (beeswax, beeswax candle, cosmetic, and textile lubricants) for various industries and provides good income for the rural population. In Botswana under normal conditions, a bee colony managed well in a top bar hive can produce between 15 kg and 20 kg of surplus comb honey for a beekeeper annually. With the prices of comb honey for farmers ranging between P40 to P50 per kilogram, this will come close to P 750 per colony per annum, therefore an apiary with 10 colonies will generate P7,500, which is equivalent to around US\$1,300. This represent an important income to an ordinary rural farmer. This research will investigate the understanding of the invaluable contribution of bees to the lives of rural communities.





## Beekeeping for Rural Development

OP-156

### Young beekeepers club: opportunity for a new beekeeping

Pablo Antonio Eduardo Maessen Bolla

Escuela Agrotécnica 4-025 Los Corralitos - Guaymallén - Mendoza - Argentina

At the 40th APIMONDIA congress held in the city of Melbourne - Australia (2007) I had the opportunity to communicate to the beekeepers gathered there the need to work on the development of beekeeping Training Centers, it was the case of the "EL CHINITAL BEEKEEPING CENTER" Space that allowed beekeepers and students to access the school for their technical training, and specialization as well as linking the agricultural schools with the beekeeping activity, taking advantage of the effort of the existence of resources belonging to public and private institutions. The project had a period of development and sustained growth until after a few years it ended up being closed for different reasons, including the lack of beekeepers in the area, as well as the growth of urban projects that made the floral fountains that supported the bees disappear. After a few years without activity, said project remained alive in the memory of many students who also left school to pursue higher studies or enter the world of work. Currently, many young people, motivated by the stories of their brothers, uncles and in some cases parents linked to the beekeeping practices that were carried out in those years, were interested in reviving the old school beekeeping project, placing the relationship of the bee with the environment as the central axis. environment and the mission to protect the *Apis mellifera*. For this reason, in the first days of December 2022, the YOUNG BEEKEEPERS CLUB of school 4-025 of Los Corralitos was founded with the aim of maintaining beekeeping as a space for learning and experimentation in agro-technical schools in the province of Mendoza - Argentina.

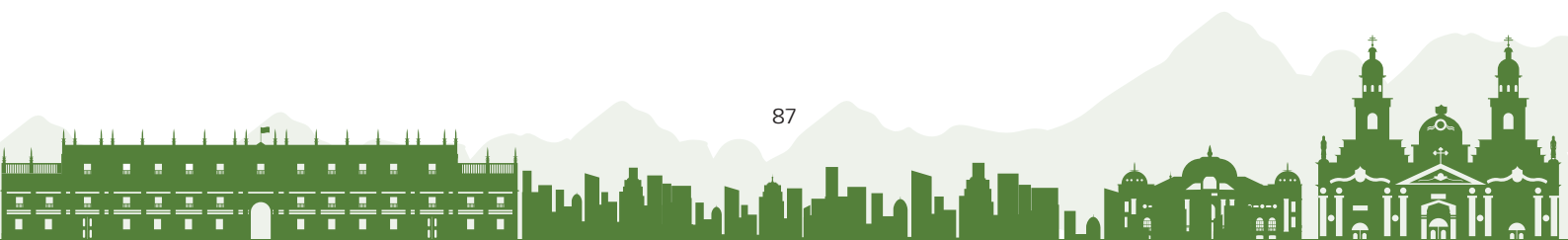
OP-157

### Raising Resilient Bees in rural pristine watershed located in Oregon, USA using closed breeding program and naturally reared queens without the use of grafting in commercial apiary family-sized operation

Eric Lewis McEwen, Joy Catherine McEwen

Adaptive Bee Breeders Alliance, member

Authors, Eric and Joy McEwen, share insights from their new book, Raising Resilient Bees, about their journey as an organic beekeeping family operation and their unconventional means of developing an inbred line of honey bees with increased levels of resistance to varroa mites. This long-term selection program when paired with management practices that include integrated pest management techniques to reduce varroa mite populations has allowed minimal use of acaricides, specifically oxalic acid twice annually. Using in-hive queen propagation techniques in lieu of grafting, daughter colonies are raised annually from every extant colony. By maintaining relatively high mite pressure, we have imposed a selective force on our inbred line toward mite resistance. Differential rearing of number of daughter colonies per over-wintered colony allows for steady genetic transformation of our metapopulation. By foregoing grafting, we prevent unintended loss of rare genetic information. We use a "soft bond" method of minimalistic intervention to maintain the population and preserve genetic diversity, by limiting genetic losses and prevent genetic bottlenecks. In eight years, our number of colonies has never gone below 250. Initial evidence suggests greater survivorship in colonies threatened by varroosis.



## Beekeeping for Rural Development

OP-158

### Re-Indigenizing Apiculture Through a Shared World-View of Interconnectedness

Melanie Kirby, Teresa Kualitay Quintana, Paul Quintana, Laurie Logan Brayshaw

Institute of American Indian Arts

There is fossil evidence dating 14 million years old of a North American native honey bee species *Apis nearctica*- found in Nevada in the United States. Though today's American honey bees are of mixed genetic origins, they are cousins to this North American ancestor honey bee. *Apis mellifera* bee subspecies present in today's American landscapes have been moved and bred across landscapes. This has encouraged hybridization between imported ecotypes/subspecies and yet, has also disrupted nuanced natural adaptations for distinct regions across the United States and neighboring countries. For many Indigenous communities across the U.S.- also referred to as Turtle Island by many North American Indigenous communities- selection and stewardship is rooted in finding and nurturing locally-adapted strains of plants and organisms. This is directly correlated to maintaining sustainability and regenerative potential. As such, the prospect of beekeeping with honey bee relatives with American Indigenous communities may also be rooted in finding locally-adapted strains. In the spring of 2021, the Institute of American Indian Arts initiated a new educational and agricultural endeavor- The Thunder Bee & Pollinator Habitat Restoration Program. They began with locally-acclimated honey bee stock which they are integrating into the Land-Grant Programs permaculture and land-based stewardship programming. Hives were installed on campus to accompany the many diverse non-*Apis* bee and pollinator species and also at several neighboring tribal communities including Taos, Santa Clara, and Cochiti Pueblos. The purpose of this integration is to demonstrate and quantify sustainable methods as based in the 4 R's of Indigenous research principles: Respect, Reciprocity, Relevance, and Responsibility. This presentation will share aspects of this process of "Re-Indigenization" in a demonstration of how interconnected reflection of how mindfulness of place and purpose can be respectfully integrated to create, maintain, and encourage sustainable and climate-adaptive approaches of apiculture for inspiring diverse livelihoods of marginalized persons and ecosystem health through biodiversity conservation.

OP-159

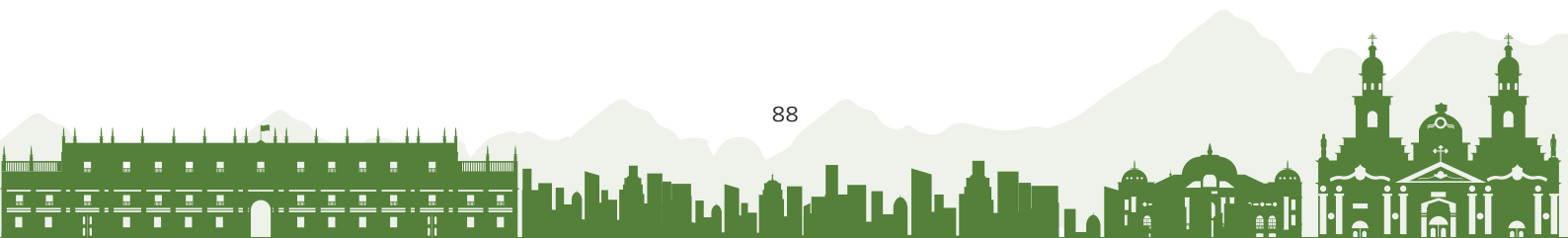
### Practical manual of beekeeping for rural areas

Javier Llaxacondor, Andrés Llaxacondor

Abejas del Perú

The relation of the Llaxacondor family and beekeeping traces back to 1957. In more than half a century this family has contributed to the development of beekeeping in Peru and other Andean countries. This manual is a synthesis of both a technical and social-environmental experience acquired while teaching beekeeping for decades. It aims at bringing meaningful content to beekeepers (especially to those in rural areas who do not have access to specialized information easily) with a practical, simple, and yet technical approach so that modern beekeeping can be understood in the more general framework of the eco-symbiotic relationship of human beings, bees and environment. Teaching beekeeping focusing on the technical and economic approach only is an error within the rural context. The main contribution of this manual is that it has been developed upon the idea that beekeeping can be an effective agent of cultural change towards environmental care and food security in vulnerable rural economies. This manual emphasizes the impact of bees in the environment and creates awareness of the human beings as being part of a wider ecosystem.

In addition to this, this manual is published in Spanish and Quechua. So it is probably the first bilingual edition of a beekeeping manual in a native South American language, enabling access to technical information to a large number of indigenous population from South America. This manual is distributed for free and it will be available online soon.



## Beekeeping for Rural Development

### OP-160

#### The One Bee Revolution

Sam Comfort

Anarchy Apiaries

Where is the fun in beekeeping anymore? Beekeeping has become muddled with gadgets, chemicals, and the rhetoric of control rather than cultivation. Historically beekeepers have always made their own queens through simple splits. This grassroots effort is once again underway and has freed beekeepers from relying on chemical varroa treatments or outsourcing special genetics. It follows bee biology and does not depend on any industrial product. The methods, founded in permaculture, are not complicated and do not have goals of saving the world. The goal is peace of mind and healthy local hives. This talk will share efforts to simplify beekeeping through a more accessible hive design - a box hive with skewer top bars called the Comfort Hive, built without power tools- and how to catch, stock, maintain, and multiply bees in these hives for free. While this road is not for everybody, it is simple enough to teach a child. It is time for beekeepers to take back the means of production.

### OP-161

#### Rural territorial development (RTD) is directly linked to the beekeeping world and its operations, needs and challenges

Patricio Nayan<sup>1</sup>, Constanza Christian<sup>2</sup>, Julio Berdegué<sup>3</sup>, Arilson Fabareto<sup>3</sup>

<sup>1</sup>Julio Berdegué, RIMISP

<sup>2</sup>Constanza Christian, RIMISP

<sup>3</sup>Arilson Fabareto, RIMISP

Rural territorial development (DTR) is directly linked to the beekeeping world and its operations, needs and challenges. This is because DTR refers to the process of improving the economic, social, cultural and environmental conditions of rural areas. With the objective of promoting economic diversification, improving the quality of life of the rural population and promoting environmental sustainability. All foundational elements of beekeeping.

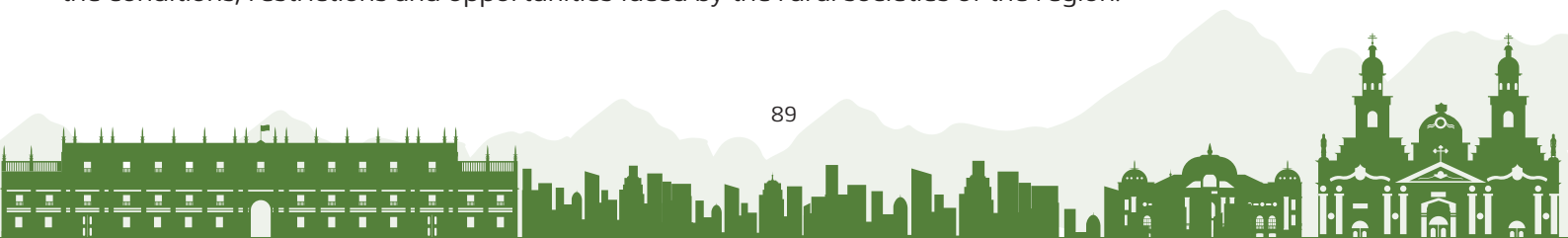
Achieving DTR requires a comprehensive strategy that involves multiple stakeholders, such as farmers, businesses, community organizations, local and regional governments, research bodies, and civil society groups.

Among the actions that can be carried out to promote DTR are:

- The promotion of sustainable agriculture and the diversification of agricultural production, including the production of organic food and the implementation of environmentally friendly agricultural practices.
- The promotion of rural tourism and the conservation of the cultural and natural heritage of rural areas.
- The improvement of basic infrastructures, such as access to water and sanitation services, electricity and transport.
- The promotion of education and training, including the training of entrepreneurs and training in business and technological skills.
- The implementation of policies and programs that promote gender equality and the social inclusion of vulnerable groups, such as indigenous peoples and Afro-descendant communities.

In summary, rural territorial development seeks to create conditions that allow rural communities to develop their full potential, in a sustainable and equitable manner. Thus, within the framework of beekeeping development, the 5 points described above are directly related to the needs and opportunities of this sector. Where the logic of the DTR takes its preponderant role of opening beyond the merely agricultural and stands as an integrating look, with sociocultural, experiential, economic, productive and finally agricultural characteristics.

The territorial approach to the development of the rural regions of Latin America continues to be the best way to face the conditions, restrictions and opportunities faced by the rural societies of the region.



## Beekeeping for Rural Development

### OP-162

#### Capacity building for beekeeping combining virtuality with Farmer Field Schools

Luis Santiago Rivas<sup>1</sup>, Enrique Bedascarrasbure<sup>2</sup>, Martín Canals<sup>3</sup>, Cecilia Dini<sup>4</sup>, Sésar Rodríguez<sup>5</sup>

<sup>1</sup>Dominican Beekeeping Network (REDAPI), Santo Domingo, Dominican Republic.

<sup>2</sup>Latin American and Caribbean Network for Beekeeping Development (REDLAC), Center of Buenos Aires Province National University (UNICEN), Argentina National Institute of Agricultural Technology (INTA), Buenos Aires, Argentina.

<sup>3</sup>Dominican Institute of Agricultural and Forestry Research (IDIAF), Santo Domingo, Dominican Republic.

<sup>4</sup>REDLAC, UNICEN, INTA, Buenos Aires, Argentina.

<sup>5</sup>Dominican Environmental Consortium (CAD), Santo Domingo, Dominican Republic.

The strategy developed is based on the capabilities of the Dominican Beekeeping Network (REDAPI) team of Dominican Republic, reinforced by researchers from the National Beekeeping Program (INTA-PROAPI) of Argentina based of 25 years of joint effort through the Latin American and Caribbean Network for the Development of Beekeeping and Family Farming (REDLAC-AF). Through the financial support of OXFAM International within the framework of the Haiti-Dominican Republic Binational Cooperation Program, it sought to combine virtual capacities, available in REDLAC from the Open Beekeeping Forum, with the experience of the Dominican team in Beekeeping Field Schools (BFS) based on the Famer Field School methodology developed by FAO. In this way, a technical updated support was achieved in an economical and efficient way, for a network of territorial technicians responsible to facilitate the BFS. The strategy implemented, allowed to combine the scientific knowledge of a powerful research and development team (25 technicians from REDAPI and INTA – PROAPI) with the experience of the beekeepers themselves, building knowledge thought learning by doing. The subjects were focused on good beekeeping practices with the participation of official, private and no governmental institutions, developing the following topics: Learning to work in the virtual classroom, Famer Field Schools methodology, Good beekeeping practices, Environment and nutrition, Health, Beekeeping management adapted to climate change, Good manufacturing practices in honey extraction and processing plants and Beekeeping diversification. 38 Beekeeping Field School sessions were held throughout the national territory, in which around 200 beekeepers and technicians participated. The plan aims to develop the scientific foundations in virtual mode by convening a highly trained team that supports territorial technicians so that they, through the BFS, promote the implementation of a technological path adapted to each region in a climate change context. The challenge is to achieve a virtual work team that allows feedback from the experiences of beekeepers, with the participation of technicians as transmission chain between researchers/ specialists and territories.

### OP-163

#### A women led-sustainable project based on meliponiculture: Sweet conservation in the Ecuadorian lowland Chocó forest

Adriana Argoti<sup>1</sup>, Citlalli Morelos-Juárez<sup>1</sup>, Yadira Giler<sup>1</sup>, Daniel Velásquez<sup>2</sup>, Sonia Bajaña<sup>2</sup>

<sup>1</sup>Tesoro Escondido Reserve Foundation, Quito, Ecuador.

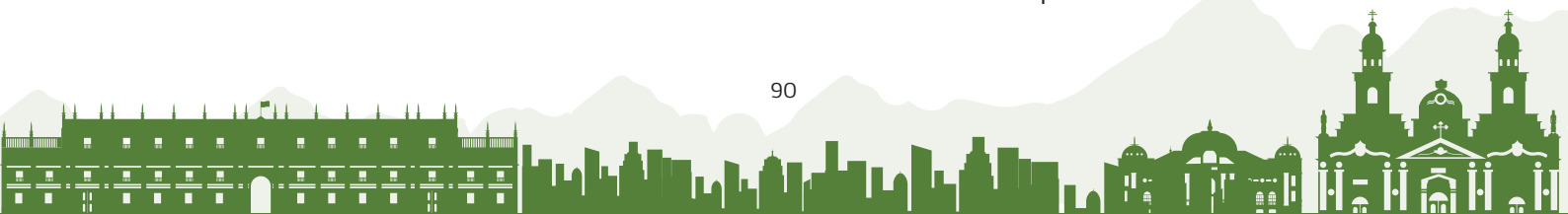
<sup>2</sup>Trainees and community members of Recinto Hoja Blanca, Esmeraldas, Ecuador.

The Ecuadorian lowland Chocó is an area with a high level of endemism within the global biodiversity hotspot 'Tumbes-Chocó-Magdalena'. It is also one of the most threatened ecosystems in the world due to deforestation, land use change and monocultures. Furthermore, the province Esmeraldas has one of the highest levels of poverty and illiteracy in Ecuador, which has a greater impact on women.

Stingless bees are among the most important pollinators in tropical forests as they are responsible for maintaining forest diversity. Deforestation, fragmentation, monocultures and indiscriminate use of pesticides threaten native bee populations, reducing numbers and potentially leading to local extinctions.

By working with people, especially women from local farming communities we enhanced knowledge of native bee populations, their importance to forest conservation and ecosystem health. During the first year of work, surveys have been conducted in seven communities to gather information on the population's knowledge of pollination and bees, three workshops were held on modern stingless bee management techniques: wooden box construction, colony transfer and disease control. At each step the attendees received technical support on their farms to ensure their success in the care and management of their colonies. The results obtained have been successful, we have healthy, strong colonies with large populations that will be ready for division this summer.

Our main objective is to empower women by establishing alternative, sustainable businesses based on native bee honey products, which are highly valued in national and international markets. The value of this project lies in its unique approach to combining conservation of one of the most diverse and threatened tropical forests with empowerment of local female farmers to lead sustainable businesses based around native meliponiculture.



## OP-164

### Sustainable development of the beekeeping industry in Costa Rica

Emanuel Antonio Miranda Cubero<sup>1</sup>, Carlos Manuel Córdoba<sup>1</sup>, José Francisco Soto<sup>2</sup>, Rodrigo Villate<sup>3</sup>

<sup>1</sup>Corona Dorada, San José, Costa Rica

<sup>2</sup>Departamento de Innovación Social, IS Corporación, San José, Costa Rica

<sup>3</sup>Programa DEVELOPPP, Agencia de Cooperación Alemana para el Desarrollo, San José, Costa Rica

Before the arrival of the Africanized honeybee (AHB) in 1984 Costa Rica exported honey to Europe, however, since the expansion of the AHB, our country became an importer of honey. Currently, all honey produced in Costa Rica is consumed locally but the national honey production is not sufficient, and we now import 50% of the honey consumed annually. The local beekeeping industry faces many adverse conditions including environmental factors such as climate change, reduced natural forage, increased agricultural acreage, and pesticide use. In addition, beekeeping associations, in general, lack generational replacement and are loosely organized, which threaten the sustainability of this productive activity.

These conditions represented a need and an opportunity to strengthen the honey value chain and produce positive environmental, social, and economic impacts. This is why in a Public Private Partnership between I.S. Corporation, Corona Dorada, and the German Agency for Development Cooperation, we develop the project "Sustainable development of the beekeeping industry in Costa Rica".

In this project four units were developed to be employed with five large beekeepers' associations in Costa Rica. The first unit was an awareness campaign that emphasizes the importance of pollination, sustainable beekeeping, and promotes the education of the consumer about hive products. This unit included the creation of an interactive App which introduced bee related topics such as pollination, biodiversity, honey quality, hive products, traceability, and market opportunities. A second unit focused on improving local environmental for honey production through the planting of 30.000 trees. This large reforestation campaign was supported and coordinated between public, private and government institutions.

Units three and four were oriented towards capacity development. In unit three, our focus was at the individual level through a "Virtual training program for beekeepers". We provided holistic coaching starting from basic bee biology, best management practices, business practices, and the future of industrialized beekeeping. These virtual courses were accompanied by face-to-face advice at the production sites. Unit four focuses on the beekeeper association, training boards of directors in organizational development principles and providing support with technical assistance in preparation for food processing and safety certification.

## OP-165

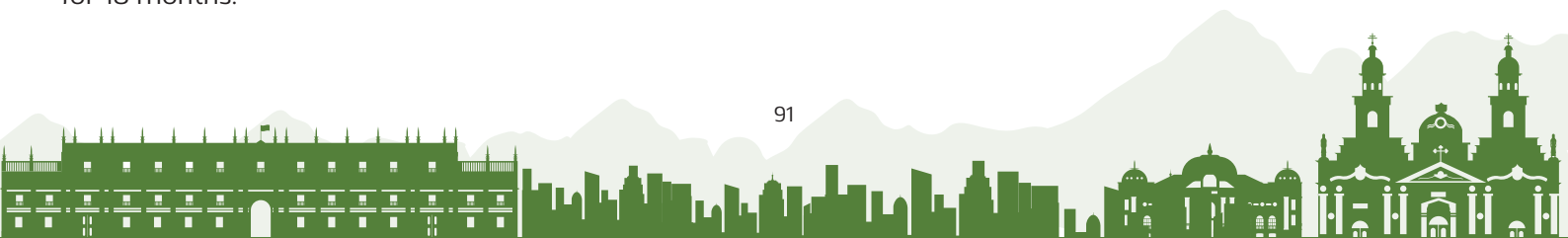
### Indigenous youth beekeeping in protected natural areas

Juan Richard Barja Ñaupari

Company: BIOAPINATIVA, Ucayali, Peru

The province of Purús has 8 native ethnic groups, each one with a unique ancestral culture and tradition, who live in harmony conserving forests and biodiversity, carrying out eco-sustainable activities. This is where our beekeeping implementation study with Africanized bees is carried out, recovered from the forests, aimed at indigenous youth within protected natural areas. In this work we present aspects such as the recovery of Africanized bees from native forests, genetic improvement, organic management, added value, eco-sustainable traceability, high-quality products and articulation of local authorities and those related to forest conservation.

With the objective of generating new alternatives for eco-efficient, sustainable production chains, especially that contribute to the conservation of bees and all the flora and fauna of protected natural areas, one of the few intangible areas in the world, thereby conserving living cultures, original ancestral traditions and profitable eco-sustainable use. The study was carried out in the Native Community of Sapote, Purús Province, Ucayali department, Peru, belonging to the Culina Madija indigenous ethnic group, with 10 young people between 18 and 30 years of age and direct assistance for 18 months.





## Beekeeping for Rural Development

OP-166

### Expanding Economic Opportunities Through Low Input Colony Propagation

Jorik Phillips

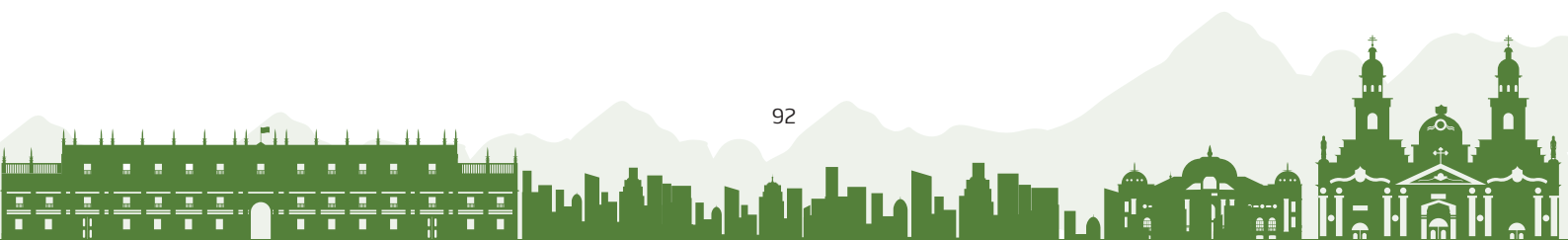
Hudson Valley Bee

Wherever you keep bees, expanding economic opportunities in beekeeping is closely linked to expanding your number of productive colonies. In this presentation, we discuss two techniques for the propagation of honeybee colonies. While this pertains primarily to how we use them in our operation in the Northeastern US, the concepts have the possibility to be used in many regions.

Technique one involves making nucleus from expanding colonies early in the season allowing them to naturally re-queen. This works best for movable frame hives including top bar hives and is easily adapted.

The second technique will cover making and using bait hives to establish new colonies This works best for all manner of hive configuration and can be used universally.

We'll discuss the most common challenges of both techniques and how to successfully overcome them.



### OP-167

#### Positioning Chilean honeys at the world market: study requested by FAO to the Pontificia Universidad Católica de Chile

Gloria Montenegro<sup>1</sup>, Ximena Ortega<sup>1</sup>, Gabriel Núñez<sup>1</sup>, Gloria Barros<sup>1</sup>, Paulina Maturana<sup>1</sup>, Claudia Giovagnoli Vicuña<sup>2</sup>, Ady Giordano<sup>2</sup>

<sup>1</sup>Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile.

<sup>2</sup>Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile.

The purpose of this study was to propose a value strategy for Chilean honeys based on differentiation to allow greater knowledge for researchers and position them in the world market. We gathered scientific evidence botanical origin and bioactive properties of honeys at national and international level. A review of the differentiation factors and the seals developed for the commercialization of honey was carried out. In addition, laboratories in Chile that specialize in the analysis to market and export the honey were reported. The quality and functionality of honey are both related to production and botanical origin. Currently, more than 30 emblematic honeys have been characterized under these standards, such as honey from New Zealand, Australia, South Korea, Japan, China, Turkey, Romania, Brazil, Spain, and Portugal. However, only two honeys from this group have developed a seal of antimicrobial activity: "Unique Manuka Factor" for Manuka Honey from New Zealand and "Total Activity" for Jarrah honey from Australia. For Chile, two types of differentiation factors on functionality and bioactivity for native honeys were described. The Active Patagonia Factor (APF) protected intellectually by the University under an Industrial Seal, later licensed to the JPM Chilean Enterprise who is now exporting Chilean honeys with added value to traditional markets and new emergent markets. This seal has received important prizes such as the London Honey Awards 2021 for Patagonia Mountain APF200+ and the Paris Honey Awards 2023 for Ulmo Rainforest APF200+, being awarded as Platinum Number 1 in honey quality in the world. On the other hand, for 15 types of Chilean honey from 3 macrozones, it was possible to collect complete information on their differentiating attributes such as botanical and geographic origin, physicochemical characteristics, bioactive compounds and antioxidant and antimicrobial activity. In based of scientific evidence of the attributes we proposed strategies to position the Chilean honeys in the world market and generate a work plan for Chile beekeeping that must involves public politics, beekeepers, exporters, researchers, accredited laboratories and the National Commission of Apiculture. Financed by FAO under the program One Country, One Priority product and by FIC proposals, CMPC proposals from Chile.

### OP-168

#### A fast, sensitive, and inexpensive test to detect fraudulent honey using mass spectrometry

Leonard Foster<sup>1</sup>, Peter Awram<sup>2</sup>, Jason Rogalski<sup>3</sup>, Sofia Colmenares<sup>4</sup>, Abigail Chapman<sup>5</sup>, Greg Stacey<sup>6</sup>, Armando Alcazar Magana<sup>7</sup>

<sup>1</sup>Leonard J. Foster

<sup>2</sup>Peter Awram

<sup>3</sup>Jason Rogalski

<sup>4</sup>Sofia Colmenares

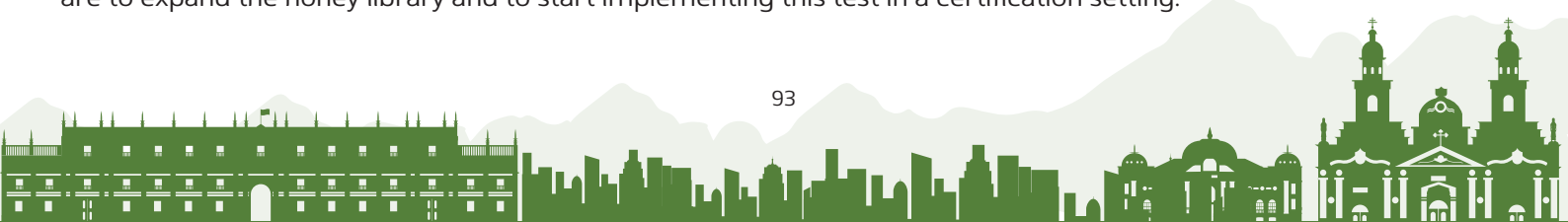
<sup>5</sup>Abigail Chapman

<sup>6</sup>Greg Stacey

<sup>7</sup>Armando Alcazar Magana

Honey is one of the most adulterated foods in the world. Syrups and other ingredients can be mixed to produce something that looks and tastes similar to honey but at a much lower cost. Passing off other lower-cost products as honey hurts beekeepers' bottom line and undermines consumers' confidence in the product, not to mention the potential health risks in consuming fraudulent foods. Various tests exist to distinguish real honey from fake but fraudsters have become increasingly inventive in circumventing those tests. The most relevant tests are the C3/C4 isotope ratio test and nuclear magnetic resonance (NMR)-based test built around the FoodScreener platform. The former is completely blind to syrups derived from C3 plants, including rice. The latter is more difficult to beat but involves several disadvantages: most importantly, the underlying database is completely closed so any suspicious results cannot be challenged or investigated. Our goals here are two-fold: 1) to develop a new method for detecting fraudulent honey based on mass spectrometry, and then to combine this with NMR data, and 2) to develop a new honey database that is both open and transparent.

Mass spectrometry is the gold standard technique for metabolic profiling and it offers orthogonal insight into samples compared with NMR. We have assembled a library of approximately 1,500 honey samples from across Canada. We have collected mass spectra on all those samples and then created a machine learning classifier to distinguish real from adulterated samples. In blind, independent tests, the classifier accurately detects fraudulent honey 100% of the time, with as little as 1% adulteration. The data also allow us to determine floral source in many cases. The next steps are to expand the honey library and to start implementing this test in a certification setting.



### OP-169

#### Honey authenticity review and method update

Christof Kunert

Eurofins Food Integrity Control Services GmbH, Ritterhude, Germany

Adulteration of honey with low-cost sugar syrups is still a widespread issue in an economically driven market with low profit margins. Highly sophisticated analytical methods are required to detect such exogenous sugar additions to honey. In 2017, Eurofins Food Integrity Control Services GmbH was the first laboratory in the world to introduce the authenticity analysis of honey by liquid chromatography, coupled with high-resolution mass spectrometry (LC-Orbitrap-HRMS) as an ISO 17025 accredited method. LC-HRMS made it possible for the first time to detect tailor-made syrups that previously remained mostly undetected in honey. LC-HRMS has become one of the leading test method in honey and Eurofins has proposed to customers a new simplified and cost-effective testing scheme for honey, the so-called "state-of-the-art"-approach, which is a combination of LC-HRMS, LC-IRMS and NMR. After five years and over 20000 successfully tested samples, we will give a honey authenticity evaluation of the last years along with the latest developments in the field of honey authenticity analysis. We will present latest fraudulent practices that have emerged on the market and how to detect them. In addition, Eurofins is the first laboratory in the world to offer a full non-target LC-HRMS analysis approach that allows the simultaneous combination of honey adulteration testing and confirmation of honey's geographical and botanical origin by LC-HRMS. This combination contributes to a significant improvement of the authenticity control of honey and demonstrates their importance on the elimination of adulterated honeys before entry into the market, thus strengthening the fair honey trade and the consumers' trust in natural high-quality products. Taken together, this makes Eurofins one of the world's leading laboratories in LC-HRMS testing of honey.

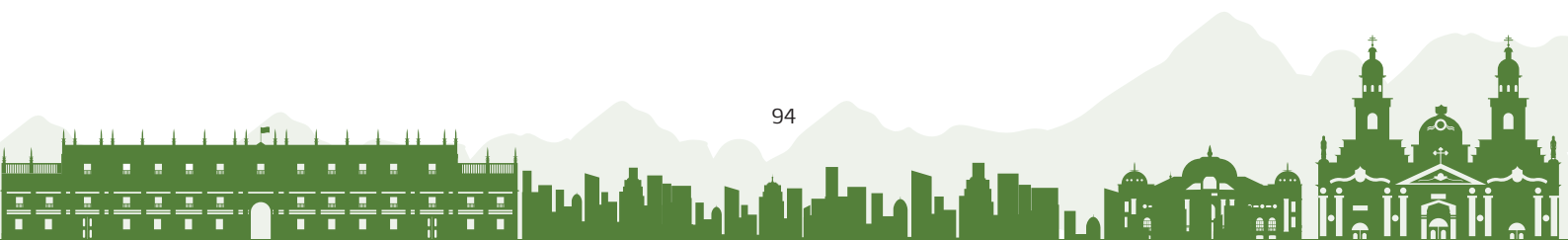
### OP-170

#### Study of quality characteristics and functionality of drone pupae (*Apis mellifera* L.)

Hyo Young Kim, Soon Ok Woo, Se Gun Kim, Hong Min Choi, Sung Kuk Kim, Seon Mi Kim, Sang Mi Han

Apiculture Division, National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea

The purpose of this study was to evaluate nutritional characterization and functionality of drone pupae. The contents of ash, moisture, carbohydrate, crude fat and crude protein in proximate composition were 4.00%, 2.14%, 15.89%, 26.19% and 51.78%, respectively. Eighteen amino acids including eleven essential amino acids were found in drone pupa with the highest level of glutamic acid at 3827.52 mg/100 g. The drone pupae containing twelve minerals was rich in K (1483.74 mg/100g) and P (714 mg/100g). In the DPPH radical scavenging assay, the ethyl acetate fraction (IC<sub>50</sub>, 559.22 ± 5.01 µg/mL) showed greater antioxidant activity than did the other samples (not detected). ABTS radical scavenging activity ranged from 170.18 to 338.86 µg/mL. The butanol fraction (IC<sub>50</sub>, 170.18 ± 11.13 µg/mL) was approximately two times stronger than that of the hexane fraction (IC<sub>50</sub>, 338.86 ± 6.84 µg/mL). The ORAC of the hexane fraction was 14.49 µM TE/g, which was significantly higher than those of the other samples. The DPP-4 inhibitory effect showed IC<sub>50</sub> values of 956.87-1491.65 µg/mL and ethyl acetate fraction showed the highest activity. These results suggest that honeybee drone pupae is a rich source of protein and other essential nutrients and thus could be used as a food ingredient and a potential candidate exhibiting antioxidant and DPP-4 inhibitory activity.



Volkan Aylanc<sup>1</sup>, Seymanur Ertosun<sup>1</sup>, Nuno Vale<sup>3</sup>, Cristina Freire<sup>4</sup>, Miguel Vilas-Boas<sup>2</sup>

<sup>1</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; REQUIMTE-LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007, Porto, Portugal

<sup>2</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>3</sup>OncoPharma Research Group, Center for Health Technology and Services Research (CINTESIS), Rua Doutor Plácido da Costa, 4200-450 Porto, Portugal

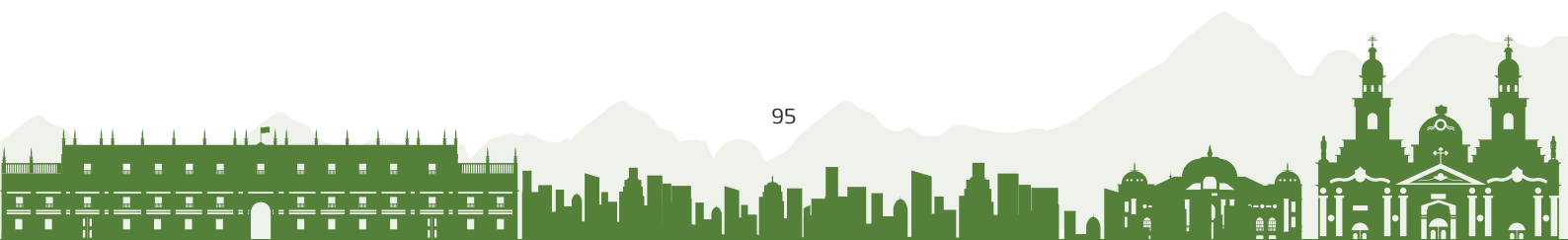
<sup>4</sup>REQUIMTE-LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007, Porto, Portugal

The manufacture and design of uniform micron-scale capsules with complex architectures for successful encapsulation of materials is a long-standing endeavor. Even though this has been achieved with the use of different materials, it carries great challenges: (i) the need of wide range of chemicals; (ii) designs of microcapsules with suitable sizes/shapes; (iii) potential toxic effects on the organism. Plant-based pollen grains are good candidates for meeting such challenges, besides being abundant and renewable resource, with species-specific architectural features, ultra-high durability, and non-toxicity.

Here, we investigate the production of microcapsules from bee pollen pellets, which guarantees microcapsules of various morphologies and sizes, quickly, with low labor-demand and reduce equipment. The palynological analysis certified the collection of different types of bee pollen pellets (*Castanea* spp., *Echium* spp., *Jasione* spp., *Papaver* spp., *Chenopodium* spp. *Helianthemum*spp. and *Cistus* spp.) with a botanical purity over 90%, separated according to their colors and collection time. The two-step extraction method was successfully applied for clearance of the hollow exine microcapsules, with nitrogen analysis revealing the removal of 79-91% of proteins that cause pollen allergic reactions. The 3D microstructure of the exine microcapsules remained intact as observed by SEM, while confocal microscopy confirmed the removal of cytoplasmic material. Laser diffraction particle sizer revealed microcapsules ranged from  $11.0 \pm 0.1 \mu\text{m}$  (*Castanea* spp.) to  $35.6 \pm 0.1 \mu\text{m}$  (*Cistus* spp.). *Chenopodium* spp. microcapsules had the highest circularity degree ( $0.93 \pm 0.01$ ) and the lowest aspect-ratio ( $1.05 \pm 0.03$ ) based on optical microscopy measurements. To understand the chemical changes in the microcapsules structure we inspected the FTIR region around  $1660\text{--}1630 \text{ cm}^{-1}$  and  $1560\text{--}1530 \text{ cm}^{-1}$ , since these vibrations have been assigned to amide I and amide II, respectively, in proteins. Those vibrations were not observed in any microcapsules. Besides, the thermogravimetric analysis showed that sporopollenin microcapsules were resistant up to  $450 \text{ }^\circ\text{C}$ .

Our findings highlight how hollow robust microcapsules with various architectural features could be easily prepared from bee pollen, opening a wide potential of applications in fields such as pharmaceuticals, food, or environmental remediation.

Acknowledgments: FCT, Portugal for funding (UIDB/00690/2020; UIDP/00690/2020; LA/P/0007/2021; 2021.07764.BD and 2021.08361.BD). Thanks to GreenHealth, Norte-01-0145-FEDER-000042.



### OP-172

#### Testing methods for honey authentication – limitations and approaches for harmonization

Klaus Beckmann

Dr. Klaus Beckmann

There has been much development on methods of analyses to detect honey fraud in the recent years, and the tests get increasingly sophisticated. The main examples are NMR, LC-HRMS and  $^{13}\text{C}$ -LC-IRMS, besides many specific tests like those to detect foreign enzymes or foreign oligosaccharides.

The complexity, however, also brings difficulties with it. More and more exceptions in the assessment of results arose due to natural occurrences of specific substances and parameters, which are usually interpreted as typical signals for additions of foreign sugars. This presentation will review the main points of discussion. Examples are the possible natural presence of the marker for beet sugar (“SM-B”), the origin of oligosaccharides or the issues with honeydew, derived from Sorghum plants in South America.

Besides the problems which naturally take place one specific issue is the fact that different labs use their own criteria and own databases to assess results of analyses. For the three mentioned methods this matter leads to varying results time to time for same honey samples in different labs.

But labs have recognized their responsibility, and approaches have been made to move together and to find solutions to minimize the number of differing results and interpretations. This presentation will describe the current state about the outcome so far.

### OP-173

#### Development and implementation of a system for the identification and location of the queen bee inside honey hives

Laura Pérez Beltrán<sup>1</sup>, Javier Fernández Caballero<sup>2</sup>, José Lorenzo-López<sup>2</sup>, Marta Cabedo Fabrés<sup>1</sup>, Miguel Ferrando Bataller<sup>1</sup>, Manuel Delgado-Restituto<sup>3</sup>, Leandro Juan-Llácer<sup>2</sup>, Karim Belhaki Rivas<sup>4</sup>

<sup>1</sup>Universidad Politécnica de Valencia (UPV)

<sup>2</sup>Universidad Politécnica de Cartagena (UPCT)

<sup>3</sup>Instituto de Microelectrónica de Sevilla (IMSE)

<sup>4</sup>Association for the innovation and development of beekeeping (INNOdeAPI)

This work presents a system for the identification and location of the queen bee in honey hives based on RFID in the 865 MHz band, without the need for visual contact. The developed system includes a micro tag that is placed on the queen bee and an external reader system.

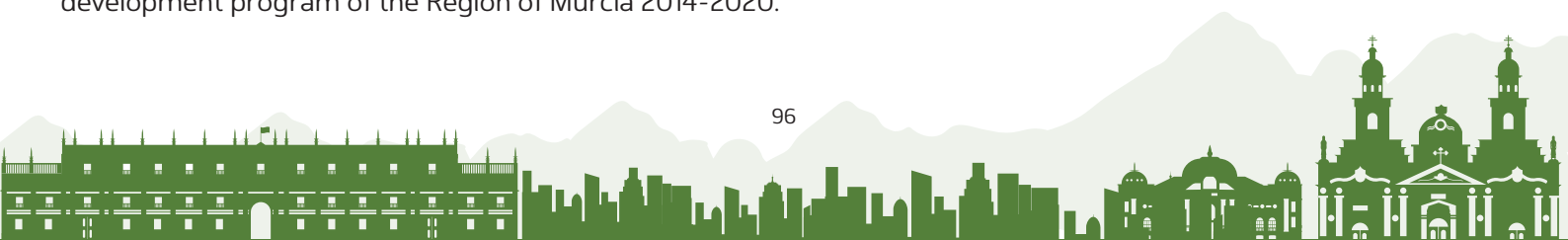
The micro tag consists of a commercial millimeter-sized transponder (microchip) and a developed micro antenna of dimensions 3.6x3 millimeters, whose design has been optimized considering the propagation environment (inside the hive). The antenna is a two-layer loop with a series capacitor for fine tuning. The substrate used is conventional FR-4 of height 0.25 mm, and two vias are used to connect the conductive lines of the loop in the upper layer and the bottom layer. The micro antenna and the capacitor have been optimized to work with the commercial transponder and to obtain the best performance in the resulting micro tag. The weight of the micro tag in the first prototypes is 0.025gr and the memory registers up to 12 digits.

The implemented external system consists of a commercial antenna and a highly sensitive RFID reader. The proposed system allows the identification and location of the queen bee at the honeycomb level. The antenna used for the reader presents circular polarization to maximize the detection of the tag for different positions of the queen bee. A two-step location-identification protocol has been developed, the first step allows to know the identification of the queen of the hive and the second one to determine on which side of the frame the queen bee is located.

The main advantages of the developed system are the reduction in the time to locate the queen bee in the routine tasks of beekeepers and the genetic traceability of the queen bees, in order to comply with the requirements in terms of animal traceability.

#### Acknowledgement

This work has been funded by the European Commission and the Comunidad Autónoma de la Región de Murcia through the European Agricultural Fund for Rural Development (EAFRD) program: Measure 16.1 of the rural development program of the Region of Murcia 2014-2020.





## Beekeeping Technology and Quality

### OP-174 Multimodal winter mortality predictor

Nico Coallier, Evan Henry

Nectar Technologies Inc, Montréal, Canada

The dependence of our food system on the availability of bees in early spring combined with the higher mortality observed in recent years leads to challenges in terms of supply and demand for pollination services. Indeed, honeybees contribute to a \$20 billion pollination industry and are essential to support our current food system. While honeybees are not dormant during winter, beekeepers can't assess the health of their colonies during these cold months which leads to high uncertainty about supply of colonies for pollination in spring. Having access to better estimates during the winter would allow beekeepers to better plan their season and would have a significant impact on the dynamics of the pollination market. Honeybees' probability to survive throughout the winter is affected by many external and internal factors. Most of the research focus has been on the internal factors and how beekeepers can supplement and/or treat their colonies in order to increase their chance of surviving. In ecology, the effect of the habitat and the phenology are often strongly correlated with survival of individuals. By combining different sources of data, we can represent external and internal factors and their relationship more accurately in our model. BeeTrack digitizes all the actions beekeepers take over the season on their hives. The geolocalisation data is highly accurate. We are able to extract satellite images on each visit timestamp. We've then created a sequence of images representing the movements and change in habitat structure throughout the season. In order to be able to also use the information logged by beekeepers which is inconsistent, we've decided to use graph neural networks (GNN) which are known to be robust in the presence of missing data. In addition, GNN are well adapted to geospatial modelling. Using a pre-trained model that we've fine-tuned on our dataset, and the sequence of actions taken on the hives, we were able to achieve 85% accuracy on a test set. You can also note that the dataset is relatively balanced. The main improvements needed are in regards to the quality of the embedding. We've used generic models to achieve our encoding.

### OP-175 Searching Africanization in Chile with a novel technique beside a certification proposal for traceability system

Andrés Vargas Fernández<sup>1</sup>, Kathleen Dogantzis<sup>2</sup>, Amro Zayed<sup>2</sup>, Brock Harpur<sup>3</sup>, Valentina Cordova<sup>1</sup>, Leonardo Porrini<sup>4</sup>, María Angélica Larraín<sup>5</sup>, Patricio Vasquez-Quitral<sup>6</sup>, Cristian Araneda<sup>1</sup>

<sup>1</sup>Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile, Avda. Santa Rosa 11315, La Pintana, 8820808 Santiago, Chile.

<sup>2</sup>Department of Biology, York University, 4700 Keele Street, Toronto, Ontario, Canada. M3J1P3.

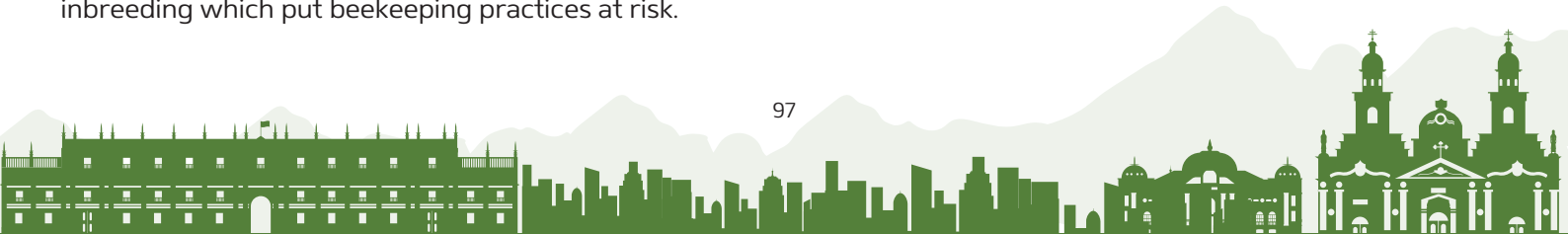
<sup>3</sup>Purdue University, Department of Entomology, West Lafayette, IN, USA

<sup>4</sup>Centro de Investigación en Abejas Sociales (CIAS), Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Mar del Plata (UNMDP), Centro de Asociación Simple CIC PBA., Mar del Plata, Argentina.

<sup>5</sup>Departamento de Ciencia de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Sergio Livingstone 1007, Independencia, 8380492 Santiago, Chile.

<sup>6</sup>Escuela de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Sede Providencia, Santiago, Chile.

Honey bees were introduced in America during the 1800' from two main lineages: Linage M (England and Spain) and Linage C (Europe), a process known as "Italization". In 1956, African honey bee subspecies *A. m. scutellata* (Linage A) were introduced to southern Brazil in an private effort to establish honey bee populations better adapted to the tropical conditions, process known as "Africanization". Africanized honey bees, expressed scutellata-like reproductive features, foraging, and defensive behavior, and spread rapidly due to their high adaptability to the tropical ecological conditions. The Africanization process involved the bidirectional flow of maternal and paternal genes between the European and Africanized honeybees (previously introduced). Due to existence of natural barriers, such as the Andes Mountain Range, the Atacama Desert and the fjords in Southern Chile, it is hypothesized that there is no Africanization in Chile. In this study, we assessed the ancestry, the genetic diversity and the population structure of Chilean honey bees by the genotyping, for the first time in Chile, of single nucleotide polymorphisms (SNP) markers. Specifically, the purpose of this work was to determine the ancestral lineage of commercial honey bees from Continental Chile (n = 39), collected between the latitudes 18°28' S and 41°28' S, and Rapa Nui Island (n=28); to compare the effects of population isolation. The results indicate that no Africanization events have occurred in Chile and that most of the current population of honey bees belongs to lineage C (includes *carnica* and *ligustica* subspecies). Honey bees genetic diversity is low both on the Continent (Ho: 0,015 and Fis: 0.308), and Rapa Nui Island (Ho:0,029 and Fis: 0,1876). Despite genetic differentiation between Continental Chile and Rapa Nui is high (Fst=0,08, p=/95%CI=), they belong to the same lineage. Applying this technique, we are able to detect and prevent Africanization events, and the excessive inbreeding which put beekeeping practices at risk.



### OP-176

#### Evaluation of the Functional Properties of the Spring Bitter Honey Collected from the Greek Island of Samothrace: Antioxidant Activity, Polyphenolic Content, Antibacterial Action and Persistence During Four Years of Storage

Panagiota Peroukidou<sup>1</sup>, Evdoxia Postali<sup>1</sup>, Eirini Maniki<sup>1</sup>, Efstathios Giaouris<sup>1</sup>, Alexandros Papachristoforou<sup>2</sup>

<sup>1</sup>Department of Food Science and Nutrition, School of the Environment, University of the Aegean, Myrina, Greece

<sup>2</sup>Department of Animal Science, University of Thessaly, Larissa, Greece

Several honey types, such as the Manuka honey, are known for their functional properties, including important antioxidant and antimicrobial actions. The present study examines the antioxidant activity (AA), total polyphenolic content (TPC), and antibacterial action of a rare honey type, the spring bitter honey (SBH) collected from the Greek island of Samothrace, which was applied in vitro in selected concentrations. The AA was determined through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and the cupric reducing antioxidant capacity assay (CUPRAC) while the Folin-Ciocalteu method was employed to determine the TPC. The antibacterial action was investigated against each one of four important pathogenic bacterial species causing foodborne diseases (i.e., *Salmonella enterica*, *Yersinia enterocolitica*, *Staphylococcus aureus*, and *Listeria monocytogenes*) using the agar well diffusion assay. SBH presented very high AA and TPC, correlated to the concentration of the samples. Their values were almost double compared to Manuka honey, used as a positive control. The comparison of annual values of samples stored in the dark under room temperatures for a four-year period, showed no differentiation during the CUPRAC and TPC test while the DPPH assay showed a better persistence of Manuka honey in time compared to the SBH. Concerning the antibacterial action, *S. enterica* was susceptible to all tested concentrations (75-6.25% v/v) of SBH, whereas samples were even more effective against *S. aureus*; in that case honey applied at 25% v/v appeared to be more effective than the antibiotic kanamycin (50 µg/µL) used to treat severe bacterial infections. *Y. enterocolitica* and *L. monocytogenes* were proven to be the most resistant of all the tested bacteria, with none of the concentrations being able to inhibit their growth. Results obtained increase our knowledge of some of the medicinal properties of SBH and may contribute to its further exploitation for health promotion and/or food-related applications.

### OP-177

#### A rare health sugar, trehalulose, discovered in stingless bee honey

Jiali Zhang<sup>1</sup>, Natasha Hungerford<sup>1</sup>, Tobias J. Smith<sup>2</sup>, Hans Yates<sup>3</sup>, Mary Fletcher<sup>1</sup>

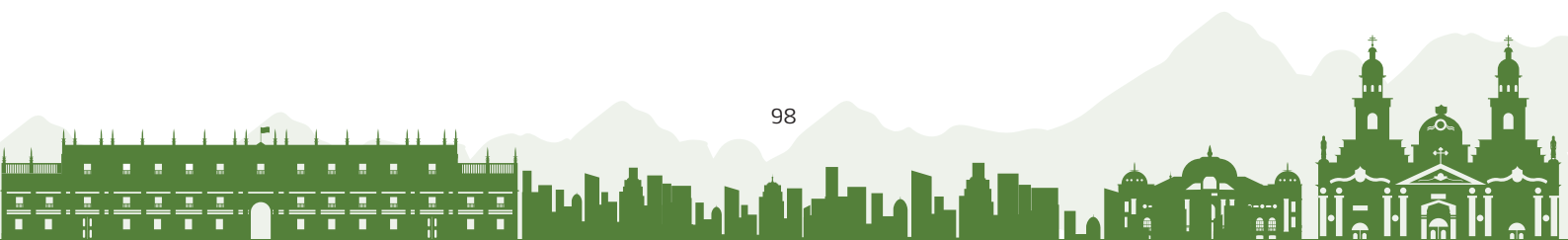
<sup>1</sup>Queensland Alliance for Agriculture and Food Innovation (QAAFI), Health and Food Science Precinct, Coopers Plains, QLD 4108, Australia

<sup>2</sup>School of Biological Sciences, The University of Queensland, Brisbane, QLD 4072, Australia.

<sup>3</sup>Forensic and Scientific Services, Queensland Health, Health and Food Sciences Precinct, Coopers Plains, QLD 4108, Australia

Stingless bee honey has long been prized for its flavour and health benefits in Australia. We discovered a rare health sugar, trehalulose, as the major sugar component (13-44%) in stingless bee honey samples from Australia, Malaysia and Brazil by liquid chromatography-mass spectrometry (LCMS) and nuclear magnetic resonance (NMR). Trehalulose is a naturally occurring isomer of sucrose with a much slower releasing rate of monosaccharides into human bloodstream. It is known to be an acariogenic and antioxidant sweetener. This study is the first identification of trehalulose as a major component within food commodity and facilitates trehalulose as the bioactive marker for stingless bee honey authentication.

The natural presence of trehalulose in stingless bee honey is highly curious. We further investigated the origin of trehalulose and gained insight into optimization of its level in stingless bee honey. By feeding sucrose solutions to confined stingless bees, the complete conversion to trehalulose (64-72%), erlose (18-23%), fructose (9-12%) and minor glucose was observed. In contrast, feeding 1:1 glucose/fructose solutions to stingless bees resulted in no conversion to trehalulose. Therefore, stingless bees are able to produce trehalulose from the isomeric disaccharide sucrose, not directly from the monosaccharide components, glucose and fructose. Hence, stingless bees with natural access to floral nectar rich in sucrose will produce honey high in trehalulose.



### OP-178

#### Molecular kit for the determination of botanical origin in Chilean honeys

**Gaspar Peña Münzenmayer<sup>1</sup>, Patricio Olavarria<sup>2</sup>, Hanile Bachmann<sup>3</sup>, Carlos Spichiger<sup>4</sup>, Luis Castro<sup>5</sup>, Kevin Valenzuela<sup>5</sup>**

<sup>1</sup>Eukarya Biotechnology, Valdivia, Chile, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

<sup>2</sup>Eukarya Biotechnology, Valdivia, Chile, Manada Chile, Valdivia, Chile

<sup>3</sup>Eukarya Biotechnology, Valdivia, Chile, Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Valdivia, Chile

<sup>4</sup>Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

<sup>5</sup>Eukarya Biotechnology, Valdivia, Chile

Chile produces honeys of high nutritional, organoleptic and medicinal quality. These properties are highly dependent on the plant species from which the bees collect the nectar. Therefore, certification of botanical origin is essential to associate properties to certain types of honey and avoid fraud. To date, the botanical origin of honey is assessed by microscopic counting of pollen grains. However, this technique is low-tech, time-consuming and relies heavily on the expertise of highly trained analysts, making it difficult to implement as a routine analysis. The objective of this study was to develop a real-time PCR (qPCR) kit to identify and quantify the main plant species present in Chilean honey. Because this technique is fast, highly specific, sensitive, and cost-effective, beekeepers will be able to efficiently analyze, manage, and certify their honeys, thus achieving better business opportunities. Here we standardized a method for DNA extraction from plant tissue and honey samples that allowed us to develop a qPCR technique for honey. We identified and sequenced 8 genomic regions that were used to design specific primers to identify the most frequent plant species in Chilean honey. Using this technical approach, we developed a prototype of a qPCR kit for ulmo (*Eucryphia cordifolia*) honey that has been used to differentiate this type of honey from other mono- and multifloral honeys of different botanical origins. In serial dilution experiments we found an inverse relationship between the cycle threshold (Ct) and the percentage of pollen. Honeys with  $\geq 45$  % pollen (Chilean standard for monofloral honeys) presented Ct values  $\leq 25$ . Honeys with  $> 80$  % pollen presented Ct values  $< 22$ , while in honeys with pollen percentage  $< 20$  % we found Ct values  $> 27$ . Taken together, these results indicate that our qPCR method is suitable for identifying plant species in honey and, therefore, could be applied to classify Chilean mono- and multifloral honeys.

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### OP-179

#### Increasing value-added of export honey by NMR fingerprints

**Paula Burdisso<sup>1</sup>, Guillermina Andrea Fagúndez<sup>2</sup>, Andrea Valeria Coscia<sup>1</sup>, María Gabriela Tamaño<sup>3</sup>, Fabricio Bauducco<sup>4</sup>**

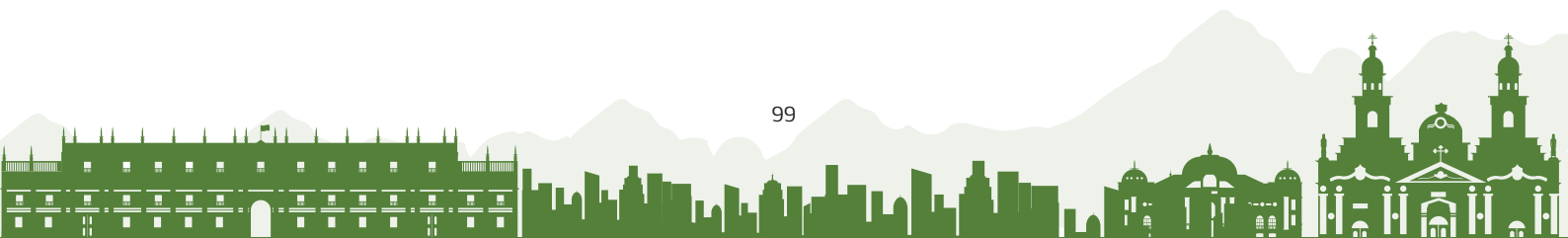
<sup>1</sup>Instituto de Biología Molecular y Celular de Rosario, (IBR-CONICET-UNR) and Plataforma Argentina de Biología Estructural y Metabolómica (Plabem), Rosario, Argentina.

<sup>2</sup>Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (CICYTTP-CONICET-UADER), Diamante, Argentina

<sup>3</sup>Laboratorio de Tecnología de la Miel y Productos Apícola (ApiUTEC), Universidad Tecnológica (UTEC), Paysandú, Uruguay.

<sup>4</sup>PROMIEL SRL, Romang, Santa Fe, Argentina.

Argentina has gained worldwide recognition for its exceptional quality honey, placing it among the top three honey producers globally. The country exports 95% of its honey products in bulk to countries that demand high-quality and safe products. To ensure the quality of this natural food, importing countries have implemented advanced analytical methods such as Nuclear Magnetic Resonance (NMR), which can identify molecular quality markers and detect adulteration in a single acquisition. In the present study, we aimed to discriminate and typify the metabolic profiles of honey samples from different ecozones in the central region of Argentina, using a combination of melissopalynological analysis and a metabolomics approach by <sup>1</sup>H NMR assisted by chemometrics. A total of 180 honey samples from four different ecoregions were analyzed. In the first step, we conducted a multivariate data analysis for the set of pollen and spectral variables separately, which successfully distinguished three of the four sample groups. Subsequently, a multi-block analysis was carried out to create a combined profile of pollen and chemical markers for each group of samples. Our results demonstrated that the multi-block analysis of pollen and spectral data is a robust analytical tool for characterizing and tracing honey from different origins. These findings provide relevant information to add value to this high-quality export product, which is critical to maintaining Argentina's position in the global honey market.



### OP-180

#### Evaluation of quality attributes, biological activity and shelf-life stability of glycolic EPP-AF® extract as a dosage form for Brazilian green propolis

Jessica Aparecida De Lima<sup>1</sup>, Giovanna Bonfanti Borini<sup>1</sup>, Andrei Felipe Moreira Buszinski<sup>2</sup>, Mikhael Haruo<sup>2</sup>, Juliana Issa Hori<sup>1</sup>, David De Jong<sup>2</sup>, Jairo Kenupp Bastos<sup>3</sup>, Andresa Aparecida Berretta<sup>1</sup>

<sup>1</sup>Research, Development and Innovation Laboratory, Apis Flora Industrial e Comercial Ltda, Ribeirão Preto/SP, Brazil

<sup>2</sup>Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto/SP, Brazil

<sup>3</sup>Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto/SP, Brazil

**INTRODUCTION:** Propolis is produced and used by *Apis mellifera* honeybees. Several biological activities have been demonstrated, mainly with the most traditional extraction process using an alcohol solution as a solvent. Although this type of solvent commonly offers the most useful chemical profile and high biomarker values, the alcohol content is considered inappropriate. Several alternative solvents have been proposed. However, the challenge is to equate the chemical profile, efficacy, and safety with that of the traditional alcohol version. **METHODOLOGY:** An EPP-AF® propolis glycolic extract was obtained by the currently and traditional extraction solvent and maceration/percolation process followed by the substitution of the alcohol solvent from the EPP-AF® alcoholic extract, by propylene glycol as carrier. Total phenolics and flavonoids were determined according to colorimetric methods using gallic acid and quercetin as biomarkers, with procedures validated according to the Brazilian agency ANVISA's guidelines. The chemical profile was also compared by HPLC and TLC procedures. Antimicrobial activity was determined using the inhibition zone model, and antioxidant activity was measured by FRAP and DPPH methodologies ( $p > 0.05$  by unpaired t-test analysis). Also, anti-inflammatory action was evaluated by measuring IL-6, IL-10, and TNF-alpha in narrow bone macrophages stimulated with LPS. The validation covered selectivity, accuracy, intra-day and inter-day precision, linearity, range, quantification, detection limits, and robustness. **RESULTS:** There was equivalent antimicrobial action against *S. aureus* and *S. epidermidis* and antioxidant activity ( $p > 0.05$  by unpaired t-test analysis). IL-6 and TNF-alpha, inflammatory cytokines, were reduced in a dose-dependent manner for both extracts, in the range of 16.7, 50, and 150 µg/mL, which were not cytotoxic for macrophage cells. The stability studies demonstrated a shelf-life of 24 months, as the biomarkers were below the maximum limit of degradation of 15%, following ANVISA regulations. **CONCLUSIONS:** The results demonstrated equivalence between EPP-AF® alcoholic and glycolic extract considering its antioxidant, antimicrobial and anti-inflammatory activities, besides equivalent shelf-life profile, suggesting that both can be used as promising ingredients for food, food supplements, pharmaceuticals, hygiene and cosmetics products.

### OP-181

#### Ionizing irradiation treatments for removing spores of *Paenibacillus larvae* preserving biological natural properties of Chilean honeys

Enrique Mejias<sup>1</sup>, Carlos Gomez<sup>2</sup>, Tatiana Garrido<sup>2</sup>

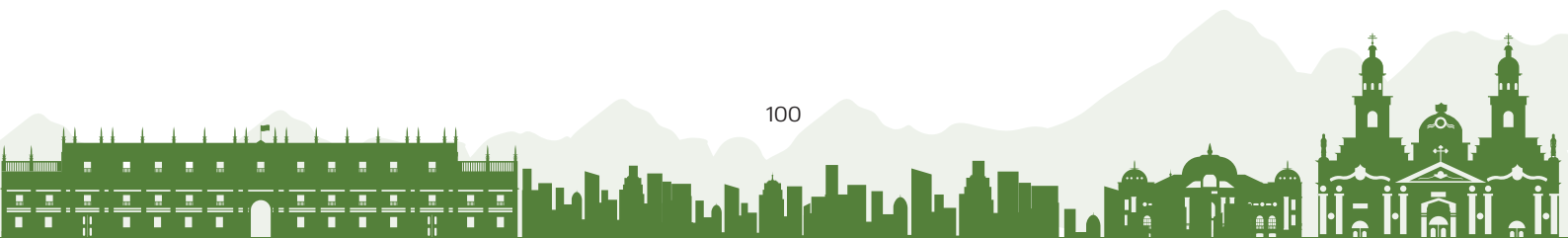
<sup>1</sup>Centro de Tecnologías Nucleares en Ecosistemas Vulnerables CTNEV. División de Investigaciones y Aplicaciones Nucleares DIAN. Comisión Chilena de Energía Nuclear CCHEN.

<sup>2</sup>Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

In Chile, honey may be produced from several native species with interesting biological properties owing to the presence of phenolic compounds inherited from specific floral sources. Chilean honeys display interesting biochemical attributes owing to its botanical origin from native and endemic species.

In recent years, the exportation volume of Chilean honeys has been increased reaching new markets with demanding regulations directed to the fulfilment of consumers expectations. In this way, there are countries with special requirements referred to *Paenibacillus larvae* spore-free honeys. This microorganism is the responsible of American foulbrood disease in beehives. Antibiotics is not allowed when an apiary tested positive to *P. larvae*. On the other hand, it is mandatory to have an accurate method to remove the potential presence of spores in the exportation bee products. Thus, Ionizing irradiation procedure can be an efficient way to achieve this goal.

In this work 56 honey samples harvested from northern, central, and southern Chile were analyzed to physicochemical patterns, total phenols, antioxidant, and anti-radical activity. Honeys with and without spores were subjected to ionizing irradiation at three levels of intensity. Afterwards, the presence of spores and the effect on phenol bioavailability, antiradical and antioxidant activities were measured again. The data shows the validation method using the experimental obtained irradiation dose for removing spores in Chilean honeys. Also, we present results of the positive correlation observed between the percentage of prevalence of native endemic species in the set of honeys analyzed and the capacity to resist this process, without altering their natural attributes determined before irradiation treatments.





### OP-182

#### Early Prediction of Beehive Survivability in Winter Using Multi-modal Sensor Data

Yi Zhu<sup>1</sup>, Mahsa Abdollahi<sup>1</sup>, Ségolène Maucourt<sup>2</sup>, Evan Henry<sup>3</sup>, Nico Coallier<sup>3</sup>, Pierre Giovenazzo<sup>4</sup>, Tiago Falk<sup>1</sup>

<sup>1</sup>INRS-EMT, University of Quebec, Montreal, Canada

<sup>2</sup>CRSAD, Canada

<sup>3</sup>Nectar Technologies Inc, Canada

<sup>4</sup>Laval University, Canada

For the past fifteen years, abnormal losses of honeybee (*Apis mellifera*) colonies have been observed throughout the world. In Canada, the estimated losses during winter are about 30% on average since 2007 despite the strict stocking management applied by the beekeepers. While existing studies have highlighted several signs in fall to identify the colonies at risk during wintering, it remains difficult to predict their survival. However, such knowledge is crucial for beekeepers to identify the high-risk colonies at the earliest stage and eliminate them before wintering to minimize financial loss. In this study, we propose some early markers that are strongly correlated with colonies winter survivability. We further developed a machine learning (ML) model to automatically detect the high-risk colonies. Our insights were obtained from multimodal sensor data (audio, humidity, and temperature) collected from 45 colonies at two different apiaries in Quebec, Canada, during a period of one full year. Metadata annotations were obtained by honeybee experts for each hive once every two weeks. To capture the underlying patterns correlated with the survivability, we extracted multiple parameters from all sensor modalities and aggregated them at different time levels (e.g., hour, day, week, and month) to perform a retrospective study. Compared to the other modalities, audio features showed higher discrimination between colonies that survived, relative to those that died during the winter. More specifically, hives that ended up dying during winter showed significantly lower average audio power between 122-519Hz and higher variation in hive power as well as humidity, and temperature. It was also noted that hives that responded differently to human interventions were associated with lower probabilities to survive the winter. We further developed an outlier detector by feeding the discriminant feature set into the Isolation Forest classifier, which achieved the Area Under the Receiver Operating Characteristic Curve (AUC-ROC) of 0.737 in identifying the colonies that failed to survive the winter. Furthermore, the interpretability and simplicity of our proposed system enable it to be deployed on low-cost hardware and allows for continuous monitoring of beehive status.

### OP-183

#### Strategies for a successful implementation of honey bee breeding programs. A look back on 30 years of hands-on-experience

Ingrid Schmaranzer<sup>1</sup>, Boigenzahn Christian<sup>2</sup>, Michael Rubinigg<sup>3</sup>

<sup>1</sup>Österreichischer Erwerbsimkerbund (Austrian Professional Beekeepers Association), Ratschendorf, Austria

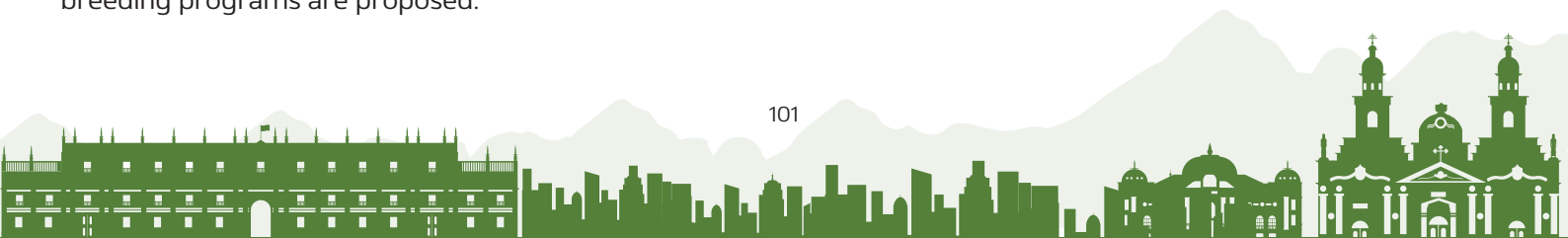
<sup>2</sup>Biene Österreich (Austrian Beekeepers Federation), Vienna, Austria

<sup>3</sup>ZAC (Consortium of Carnica Breeders), Linz, Austria

Professionally managed breeding programs are designed to increase the productivity of honey bee colonies but might also help to adapt honey bee populations to changing environmental conditions, reduce the susceptibility to diseases, provide resilient and easy-to-manage livestock and play a crucial role in the preservation of populations of *Apis mellifera* worldwide. Launched in 1992, the Austrian honey bee breeding programme is the world's longest continuously run programme in which selection is based on estimated breeding values. Currently, two populations are bred in this programme, Carnica and Mellifera.

An evaluation of the Carnica breeding program shows that a low but statistically significant genetic gain could be achieved for all performance traits tested in the population (e.g. an average increase of 1.3 % per year for the weighed total estimated breeding value). The average inbreeding coefficient is low (average of 0.026), indicating only a moderate decrease in genetic diversity. The real value of this evaluation, however, lies in the comparison of the theoretical requirements and the practical feasibility of measures necessary to achieve a substantial genetic gain for a given trait: (a) the necessity to conduct field tests performed by beekeepers and the limited economic value of breeding animals, limiting the use of performance test methods; (b) low testing rates, resulting in low selection intensity and suggesting that significant genetic improvement, in particular of traits with low heritability, is unlikely to be successful.

Opportunities, but also organisational, economic and technical challenges in the practical implementation of such programs (structuring of performance tests, testing rates, data quality, herdbook management, mating control) are discussed. Costs incurred by the implementation of such programs are compared with potential economic benefits. Building on this hands-on-experience of more than 30 years, strategies for a successful implementation of honey bee breeding programs are proposed.





### OP-184

#### Lactic acid bacteria isolated from *apis mellifera* and their ability to convert trans-10-hydroxy-2-decenoic acid in royal jelly to 10-hydroxydecanoic acid

Hayate Itatani, Ayanori Yamaki

Institute for Bee Products & Health Science, Yamada Bee Company, Inc., Okayama, Japan

The major fatty acids in royal jelly (RJ) are trans-10-hydroxy-2-decenoic acid (10H2DA) and 10-hydroxydecanoic acid (10HDAA). 10HDAA from RJ has been reported to support M-cell differentiation and induce antigen-specific immunoglobulin A (IgA) in macaques, and is expected to be an immunostimulatory food ingredient. Since the content of 10HDAA in RJ is about one-third of that of 10H2DA, if 10H2DA in RJ can be converted to 10HDAA, the immunostimulatory capacity of RJ can be increased. In this study, we searched for bacteria derived from honeybees that convert 10H2DA to 10HDAA, and attempted to produce RJ with a higher ratio of 10HDAA (fRJ). Honey stomachs and intestines of worker bees and queens (*apis mellifera*) were collected, and after selective culture on TOS propionate agar medium, Gram staining and catalase reaction tests were performed. The above bacteria were added to MRS medium containing 10H2DA and cultured, and the rate of conversion to 10HDAA was determined by HPLC. The 16S rDNA sequences for the strains with conversion ability were compared to the homology rate with standard strains. We isolated 157 bacteria strains from Honey stomachs and intestines. Two strains from intestines of queen bee (QB 3-2-4, 9-1-6) were capable of conversion to 10HDAA. QB 3-2-4 and 9-1-6 were identified by BLAST analysis. 16S rRNA sequences as sharing 99.7% identity with *Lactobacillus panisapium* Bb2-3, and were named M1 and M2. This is the first report of the discovery of lactic acid bacteria that converts 10H2DA to 10HDAA. We plan to evaluate the effect of fRJ as an immunostimulatory food in human clinical trials.

### OP-185

#### Application of non-targeted LC-HRMS in routine analysis

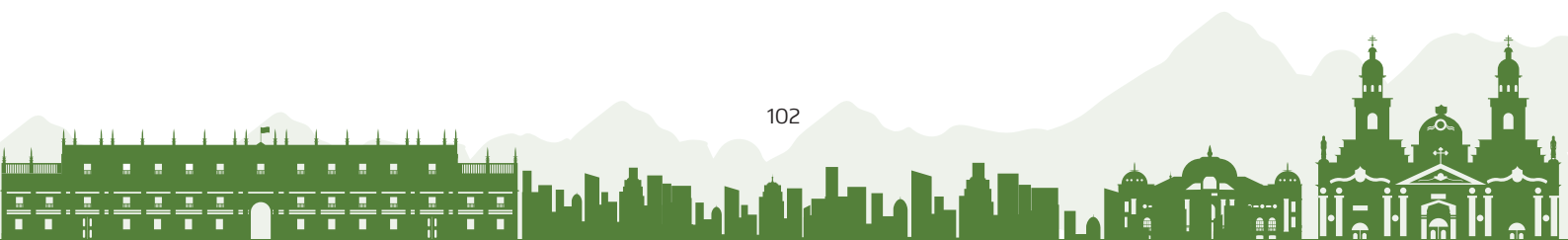
Jule Hansen<sup>1</sup>, Christof Kunert<sup>2</sup>, Stephan Seifert<sup>1</sup>

<sup>1</sup>University of Hamburg, Hamburg, Germany

<sup>2</sup>Eurofins Food Integrity Control Services GmbH, Ritterhude, Germany

In routine analysis, the highly sensitive method of liquid chromatography high-resolution mass spectrometry (LC-HRMS) is currently used for the detection of pre-defined marker signals. When used to test the authenticity of honey, these markers reliably indicate different syrups added or mislabelling of botanical or geographical origin. However, the disadvantage is that it only detects known counterfeits for which marker molecules have been defined. Since the fraud methods are constantly being adapted to the latest advances in routine analysis, the targeted approach therefore requires that marker databases are constantly updated and completed. For commercial laboratories to stay ahead in this race, it makes sense to use non-targeted methods because they can even detect unknown adulterants. This is possible because the entire set of detected signals is analysed, which is why non-targeted LC HRMS data are very complex and machine learning techniques are applied for the extraction of useful information. These algorithms use training data, for example data with a known geographical origin of pollen, and train a model on the differences between the predefined classes. This model can subsequently be applied for the classification of other samples, e.g. with unknown or questionable origin. Since a commercial laboratory has many authenticated samples, the ideal conditions are given to obtain powerful machine learning models.

The reason why untargeted LC-HRMS is currently not used in routine analysis is the lack of suitable data processing strategies, which enable long-term comparison of samples analysed on different instruments and at different time points. We have developed an approach that meets the needs of routine analysis and provides robust and reproducible results. In this presentation we will show the first results and demonstrate that our approach enables the long-term application of untargeted LC HRMS for honey analysis.



### OP-186

#### **“Development of a sustained release, high absorption and stable system based on microencapsulated green propolis standardized extract (EPP-AF®) – i-CAPS”**

**Andresa Aparecida Berretta<sup>1</sup>, Soraia I Falcão<sup>2</sup>, Ricardo Calhelha<sup>2</sup>, Lilian Barros<sup>2</sup>, Jéssica Aparecida De Lima<sup>1</sup>, Nathaly Alcazar Amorim<sup>1</sup>, Isabela Salgado Gonçalves<sup>3</sup>, Hernane Da Silva Barud<sup>3</sup>, Jairo Kennup Bastos<sup>4</sup>, David De Jong<sup>5</sup>, Miguel Vilas Boas<sup>2</sup>**

<sup>1</sup>Research, Development and Innovation Department, Apis Flora Indl. Coml. Ltda., Ribeirão Preto, Brazil

<sup>2</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

<sup>3</sup>Biopolymers and Biomaterials Group, University of Araraquara, Araraquara, Brazil

<sup>4</sup>School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

<sup>5</sup>Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

Propolis is produced by honey bees (*Apis mellifera*) to protect the hive and its inhabitants. There is great interest in this natural material for use in health products; however, due to its low water solubility, propolis generally has low bioavailability. Its most common form of presentation, an ethanolic extract, also has the disadvantage of containing alcohol and having a strong taste. An alternative, lyophilized propolis, has the drawbacks of high hygroscopicity and instability. There is a need for alternative formulations with increased solubility, while remaining safe and stable. Propolis-loaded microcapsules were prepared with spray-dryer technology from an emulsion made with green propolis (EPP-AF®) extract and acacia gum (40:60). These propolis-filled microcapsules were characterized using HPLC-DAD, quantified using external standards of p-coumaric acid, artemillin C, along with determination of total bioactives, MEV, % efficiency of encapsulation, delivery profile using a Franz diffusion cell, evaluation of absorption in CaCO<sub>2</sub> in vitro cell culture, besides a chemical 24-month shelf-life profile. Spherical propolis-loaded microcapsules were obtained, with 93.7% encapsulation efficiency. The chemical fingerprint was identical to that of the original hydroalcoholic propolis extract used in the production system. After 24 months under shelf-life conditions, key components were reduced only 2.7% for p-coumaric acid, 14.2% for artemillin C, and 7.8% for total bioactives, which is quite good considering the high instability of artemillin C when exposed to warm temperature and light. Delivery of p-coumaric acid from the microcapsules in cell culture reached 40% at 8 hours, while artemillin C demonstrated a longer sustained release, attaining 30% at 72 h. The kinetics calculation demonstrated that the microcapsule system followed a zero-order model. The cell culture absorption model showed that the propolis-loaded microcapsules are better absorbed than lyophilized propolis extract. Propolis EPP-AF® microcapsules (i-CAPS) provide a sustained release system with a stability profile within the maximum degradation limits approved by the Brazilian National Health Surveillance Agency (ANVISA) guidelines (<15%), and consequently are a promising candidate for substitution of hydroalcoholic propolis extract.

### OP-187

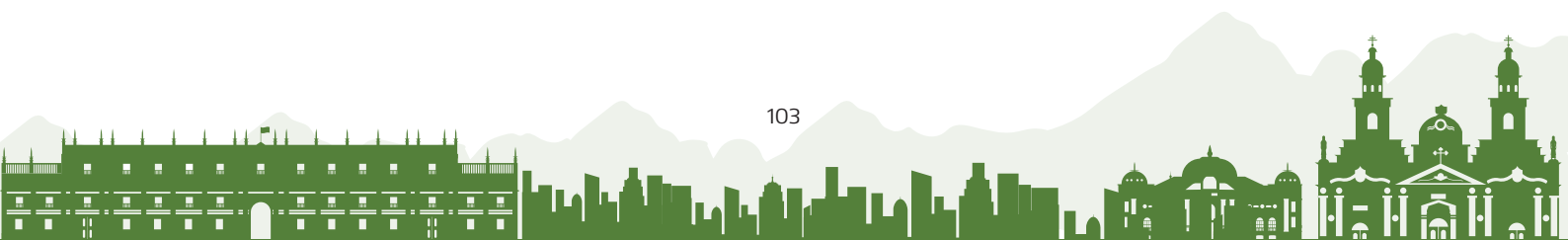
#### **An IT-based Group-enabled Incubator for Queen Bee Rearing with Immediate Hatching Notification**

**Andree Hildebrandt, Oliver Theel**

Department of Computer Science, Carl von Ossietzky University, Oldenburg, Germany

For successful beekeeping, queen bees with good characteristics are very important. For rearing high quality queen bees, many techniques are known. Some of those techniques rely in some stage on the use of incubators. An incubator takes care of an already capped queen cell until hatching. During this period, the incubator guarantees a brood nest-like environment by stabilizing temperature and air humidity. Incubators typically cage queen cells one-by-one in order to prevent hatched queen bees to kill each other. Unfortunately, once hatched, the incubator ceases to provide an almost ideal environment for the queen bee: from now on, the queen bee requires being fed and taken care of by other worker bees. Thus, a hatched queen should immediately be taken out of the incubator and put into a hive. Although nowadays available incubators succeed in providing stable living conditions temperature- and air humidity-wise, they fail to optimize queen bee rearing right after hatching, since it is up to the beekeeper, to "manually" learn about the hatching event. This is basically done by personally inspecting the queen cells once or twice a day: a cumbersome and -for beekeeper as well as animal- sub-optimal procedure.

We therefore designed and implemented a novel incubator that besides controlling temperature and air humidity allows for immediate queen hatching notification via smartphone. Hatching is detected by a changed pattern of optically detectable movement in a cage of a supervised queen cell. The incubator supports multiple series of queen cells in different development stages. For every series, different beekeepers can automatically be notified if their particular queens have hatched, thus, allowing convenient sharing and operation of the incubator among some group of beekeepers. The incubator offers additional management features, such as notifications of temperature and air humidity deviations from optimal set points. Beekeepers can be assigned different roles with varying capabilities when using the incubator for restricting flawed configuration or operation. Furthermore, the incubator logs and persists all relevant data for graphical on-the-fly or ex-post analysis and inspection, thereby rendering the incubator-based rearing period highly traceable such that the incubator supports optimized and quality-insuring queen bee rearing.



### OP-188

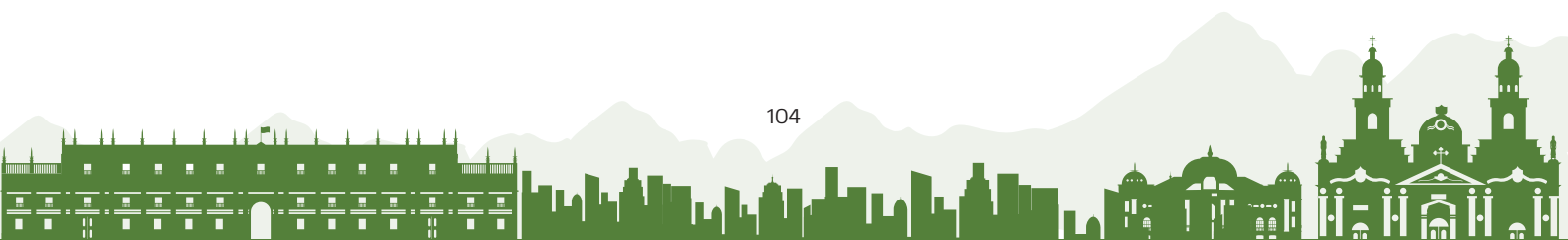
## “Propolis International Standard Proposed by ISO – an important regulatory framework for authenticity and quality requirements of the international propolis market”

Andresa Aparecida Berretta<sup>1</sup>, Miguel Vilas Boas<sup>2</sup>

<sup>1</sup>Research, Development and Innovation Department, Apis Flora Indl. Coml. Ltda., Ribeirão Preto, Brazil, Convenor of ISO/TC34/SC19/WG2 Propolis, President of ABEMEL (Brazilian Association of Companies and Exporters of Bee Products), Rio Claro, Brazil.

<sup>2</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal, Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Bragança, Portugal

Propolis is a resinous material produced by bees to protect their hives. Bees collect bioactive exudates and resins of plants available on the apiary surroundings that they select to make propolis. Consequently, due to variations in plant species in the different regions of the world, propolis types are very diverse in their organoleptic and chemical properties, with a great range of biological activities. Nowadays, the main types of propolis traded are brown from Poplar (*Populus* spp.), produced mainly in Europe and Asia, followed by green propolis from *Baccharis dracunculifolia*, exclusively from Brazil, and red propolis from *Dalbergia* and *Clusia* spp. produced in Central and South America. The current global supply of propolis is estimated to be 700-800 ton/year, with an market value of US\$ 700 million/year, forecasted to reach US\$ 829 million by 2027. However, a major challenge for this market has been a lack of standardization among the companies that commercialize it, besides a lack of regulations in most countries. There is an urgent need for internationally validated and standardized methodologies, and for the establishment of authenticity and quality requirements. In this scenario, the project ISO/NP 24381 initiated in 2019 at ISO, is an important regulatory framework. This project was constructed by experts from 17 countries around the globe. The document specified quality requirements, analytical methods, packaging, marking, labelling, as well as storage and transportation conditions for propolis produced by *Apis mellifera* bees. The methodologies proposed were inter laboratory validated by 14 laboratories around the world in Belgium, Brazil, China, German, Italy, France, Portugal, Romania, Spain, and Turkey, with seven samples tested for evaluation: 5 brown, 1 green, and 1 red propolis. Eight analytical parameters were determined for the purposes of this standard: ethanol extractables determination, total ash, petholeum ether extractables, loss on drying, total phenolics and flavonoids, phenolic profile, and antioxidant capacity. This document will probably be the first regulatory framework published around the world establishing the background for authenticity and quality requirements for propolis. Thanks to the experts of ISO Working Group 2 (TC34/SC19-WG2-Propolis) for this important contribution in the field.



## Beekeeping Technology and Quality

### OP-189 Honey traceability concept using blockchain technology

Lucian Avramescu

University of Life Sciences "King Michael I" from Timisoara

As the global demand for honey continues to rise, there is a growing concern about the authenticity and traceability of honey products. Honey is a valuable commodity that is susceptible to adulteration, and it can be difficult to trace the origins of the product. The use of blockchain technology could provide a solution to this problem.

Blockchain technology is a distributed ledger that allows for secure and transparent tracking. By using blockchain technology, honey can be traced from the hive to the consumer, ensuring that the product is genuine and has not been tampered with.

The honey traceability process could work as follows: Each beekeeper would be assigned a unique identifier, which would be recorded on the blockchain. When the honey is harvested, it would be assigned a unique identifier as well. This identifier would be linked to the beekeeper's identifier and recorded on the blockchain. The honey would then be transported to a processing facility, where it would be assigned another unique identifier. This identifier would be linked to the previous identifiers and recorded on the blockchain.

At each step in the process, the blockchain would record the date, time, location, and any other relevant information. This information would be accessible to everyone on the blockchain, ensuring transparency and accountability. The blockchain could also be programmed to automatically verify the authenticity of the honey, based on the information recorded.

The use of blockchain technology for food products traceability has already been explored by several companies, including Walmart, which has implemented a blockchain-based traceability system for its mangoes and pork products. The technology has also been used in the wine industry, where it has been successful in preventing fraud and ensuring the authenticity of the product.

In conclusion, the use of blockchain technology for honey traceability could provide a solution to the problem of authenticity and traceability in the honey industry. By recording each step in the process on a transparent and secure blockchain, honey can be traced from the hive to the consumer, ensuring that the product is genuine and has not been tampered with.

### OP-190 Study of natural constituents, contaminants, and adulterations in honey by metabolomic analysis

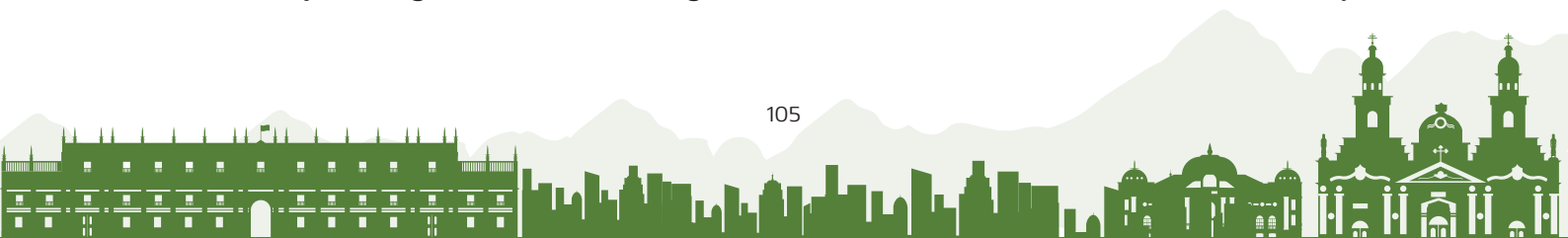
Lady Viviana Camargo Ovalle<sup>1</sup>, David Guillermo Piedrahita Marquez<sup>2</sup>, Marcelo Maraschin<sup>3</sup>, Aline Nunes<sup>3</sup>, Consuelo Díaz-Moreno<sup>1</sup>, Hector Suarez<sup>1</sup>

<sup>1</sup>Departament Faculty Agricultural Sciences, National University of Colombia, Bogotá, Colombia.

<sup>2</sup>Departament Faculty Sciences, National University of Colombia, Bogotá, Colombia.

<sup>3</sup>Departament of Fitotecnia, Faculty Agricultural Sciences, Federal University of Santa Catarina. Florianopolis, SC, Brasil.

One of the most significant advances in biotechnology and food science and technology to identify bioactive compounds has been the use of high throughput analytical techniques such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) coupled to computational tools, i.e., bioinformatics, for chemometric analysis, allowing to chemically profile complex matrices such as floral honeys, for instance. MS and NMR spectroscopy enable the detection, and quantification of primary and secondary metabolites with functional properties in food matrices, as well as detecting contaminants or adulterants and obtaining toxicological profiles in quality control processes. In this sense, metabolomics studies applied to different bee products (honey, pollen, propolis, wax, and royal jelly) present great potential to the market, due to the ability to identify new functional compounds, the geographical origin for traceability purposes (e.g.), as well as to detect adulterations in samples. Due to the chemical complexity of bee products and the efficiency in characterizing them of metabolomics, it is extremely important to include this analytical approach for the characterization of profiles and identification of adulteration in honeys. Thus, this review aimed to elaborate a compilation of the scientific information available that will allow one to observe the evolution of the state of the art in this subject and give to the audience a general outline of the advances in food omics in honeys.



### OP-191

### Comparison of different sizes of mating boxes in Langstroth and Dadant hives in mating and performance of produced queens

Leonidas Charistos, Michalis Moutsakis, Fani Hatjina

Department of Apiculture, Inst. of Animal Science, ELGO 'DIMITRA, Greece

In queen rearing procedure, the size of the mating boxes play an important role for the successful mating, also care of the queens and at the same time determines the capacity of the queen breeder to populate these micro-colonies. In Greece, the queen producers are using different sizes but there is no real data on the mating success of the queens as well as on the acceptance rate late on. Similarly, there are unanswered questions on the performance between the Langstroth and Dadant hives in our climatic condition. Therefore 3 different sizes of mating boxes occupied 1/2, 1/4 and 1/8 of Langstroth and Dadant hives were used to mate the queens, which later were introduced to normal Langstroth and Dadant hives to be monitored for their performance. The results showed that the mating success was higher in the mating boxes equal to 1/2th of the hive, followed by the 1/4th and the 1/8th (80%, 65% and 45% respectively). The initial acceptance rate was similar between Dadant and Langstroth hives but it was higher on the queens mated in the 1/2th and 1/4th compared to the 1/8th mating boxes (100% and 90% respectively). Furthermore, the follow-up of the queens in the respected hives, showed that colonies in Dadant hives tend to keep higher no of bees and brood, also collected higher amounts of honey, but they also had a higher swarming tendency. Higher number of queens were finally accepted in Langstroth hives, and the lower number of them was originated from the mating boxes equal to 1/4th of the hive. We conclude that at least in our own conditions, Dadant hives are as good as the Langstroth hives and when queens are mating in the mating boxes equal to 1/2 of the hive are performing better.

### OP-192

### The methodology and monitoring of honey bee food supplements and substitutes

Michel Bocquet<sup>1</sup>, Soraia Falcão<sup>2</sup>, Alessandra Giacomelli<sup>3</sup>, Róbert Chlebo<sup>4</sup>, Maja Ivana Smodiš Škerl<sup>5</sup>, Giancarlo Quaglia<sup>6</sup>

<sup>1</sup>Apimedia, 82 Route de Promery - Pringy - 74370 ANNECY, France

<sup>2</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>3</sup>UNAAPI, Via Paolo Boselli 2, 50136 Florence, Italy

<sup>4</sup>Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

<sup>5</sup>Agricultural institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia

<sup>6</sup>Lifeanalytics- FLORAMO, Via Lime 4 - 12047 Rocca De' Baldi (CN), Italy

The nutritional status of a honey bee colony is recognized as a key factor in ensuring a healthy hive. A deficient flow of nectar and pollen in the hive immediately affects its development, making room for pathogen proliferation and, consequently, for a reduction in activities and strength of colony. It is therefore urgent for the beekeepers to use more food supplements and/or substitutes in the apiary management, allowing to address honey bee nutritional imbalances according to the beekeeper's desired results. In this context, the commercial market for beekeeping products is growing rapidly due to low regulation of animal food products and of the beekeeper's willingness to guarantee healthy colonies.

There are numerous products (supplements and substitutes for honey bee colonies) currently available on the worldwide market, with a highly variable and sometimes even undefined composition, claiming a set of actions at the level of brood stimulation, energy supplementation, queen rearing support, reduction of varroa reproduction levels, improvement of the intestinal microflora of bees, improvement of the health of hives infested by pathogens.

To address this issue, the members of COLOSS (Honey Bee Research Association), NUTRITION Task Force, for the first time propose an action on honey bee feed control and monitoring, setting the four main OBJECTIVES:

- 1) Elaborate methodologies to study bee aliments (protocols, good laboratory practices), and to implicate different stakeholders to clarify the type of analyses depending on their needs (e.g. organic or legal framework);
- 2) Create and coordinate a network of laboratories able to use the proposed methodologies (ring tests, evolution of the methods with new technologies);
- 3) Apply the methodologies to a large set of bee aliments at a worldwide scale; and
- 4) Elaborate guidelines to support and assist food companies and regulators to proceed the effective control of the quality and safety of supplements and substitutes for honey bees.

Globally, this action will allow more information to the stakeholders via monitoring and set the basis for regulation of products to reach the minimum standards for quality, effectiveness, and economy of honey bee feed, and finally guaranteeing the quality of the bee products.





### OP-193

#### Artificial Intelligence to count and discriminate honey bee activity at the hive entrance

Raquel Martín Hernández<sup>1</sup>, Soledad Sagastume De Andrés<sup>2</sup>, Mariano Higes Pascual<sup>2</sup>, Antonio Nanetti<sup>3</sup>

<sup>1</sup>Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA-IRIAF), PCTCLM-INCRECYT (co funded ESF/FSE); Marchamalo, Spain

<sup>2</sup>Centro de Investigación Apícola y Agroambiental de Marchamalo (CIAPA-IRIAF), Marchamalo, Spain

<sup>3</sup>Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca inCentro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA); Bologna, Italy

The study of bee foraging activity under different levels of environmental stress is one of the most ambitious challenges. Within the MEDIBEES project, one of the objectives is to compare foraging activity in several Mediterranean countries, so it is necessary to carry out field trials to determine their activity and to make these comparisons reliable. The most commonly used method so far has been to manually count the bees entering and leaving the hive during a given time. Ideally, several measurements should be taken at different times of the day throughout the beekeeping period. However, this method is time-consuming and the number of measurements is usually small, which can lead to inaccurate data on bee activity. On the other hand, colonies in very warm environments maintain their optimal temperature conditions because the bees remain static at the entrance, beating their wings to increase the airflow inside the hive. At CIAPA in Marchamalo (Spain), a computer tool developed by the Spanish company K.R.C. Española S.A., (<https://www.krc.es>) has been tested which, by means of artificial intelligence, has managed to count with more than 96% reliability both the bees that enter and leave the colony and those that remain static at the entrance of the hive. This tool works on high quality videos of about 2 minutes previously recorded from the hive entrance and can be programmed to collect several videos, with the added advantage that data can be collected from several hives at the same time, allowing for a more accurate comparison. This new device represents a breakthrough in the study of bee colony activity in real time.

MEDIBEES is part of the PRIMA program supported by the European Union

### OP-194

#### Application of Fluorescence Spectroscopy for the Detection of Adulterated Honey in the UK

Alex Rozhin<sup>1</sup>, Raghavan Chinnambedu Murugesan<sup>1</sup>, Lynne Ingram<sup>2</sup>, Steven Daniels<sup>1</sup>, Valentina Barker<sup>1</sup>

<sup>1</sup>Aston Institute of Photonic Technologies College of Engineering and Physical Sciences Aston University, Birmingham, UK

<sup>2</sup>Honey Authenticity Network UK

Honey is a high-value product, but in recent decades, there has been a significant increase in honey adulteration and fraud. UK beekeepers are particularly concerned about the quality of honey supplied, as in the recent EU study "From the Hives" all samples from honey consignments exported from the UK were flagged as suspected of being adulterated [1]. Existing testing methods, such as chromatographic methods, sensory analysis, pollen study are either becoming obsolete or are expensive, time-consuming, and required highly trained personnel with sophisticated lab equipment.

Fluorescence (FL) spectroscopy has the potential to make honey testing more agile, quicker, and more widely available [2]. The method uses a spectral "fingerprint" of bio-chemical components in honey and can detect tampering, contaminants, and even the geographical origin of honey based on the presence of native plant chemicals. This method has already been proven effective and can complement existing methods.

In this report, we present fluorescence excitation-emission spectroscopy data of various honey samples produced in Britain. We aim to create a standard FL spectroscopic database to determine principal chemical components, such as polyphenols, furosines, and other Maillard products present in various honey samples. The changes in FL spectral features, such as peak position, intensity, and peak shapes will be used to authenticate the honey standard. By using FL spectroscopy linked with machine learning data analytic methods, we hope to provide a faster and more accessible method for honey testing that can help maintain the integrity of the UK honey industry.

#### Collaboration

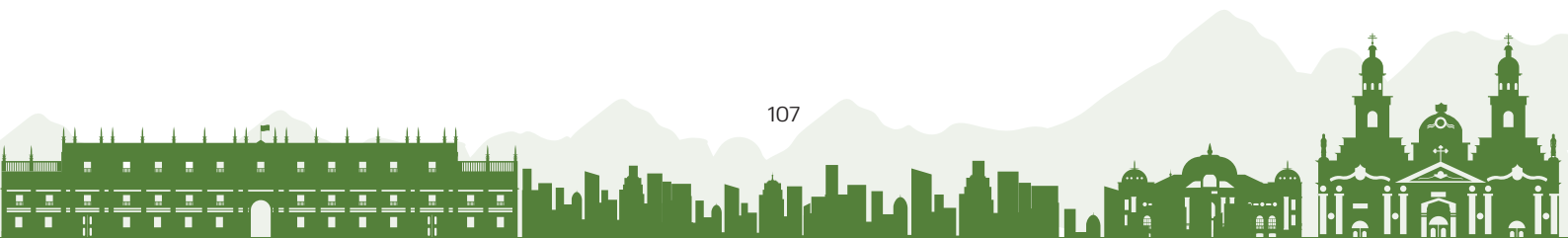
This project is a collaboration between Honey Authenticity UK and Aston Institute of Photonic Technologies at Aston University and is supported by BBKA. We intend to work with key industry stakeholders and regulators to develop honey samples database and authentication protocol.

#### Reference

[1] EU Coordinated Action "From the Hives. Sampling, investigations and results. European Commission, 2023.

[2] L. Lenhardt et al. / Food Chemistry 175 (2015) 284–291.

Globally, this action will allow more information to the stakeholders via monitoring and set the basis for regulation of products to reach the minimum standards for quality, effectiveness, and economy of honey bee feed, and finally guaranteeing the quality of the bee products.



### OP-195

#### Using an updated Regional Climate Model in combination with beekeeping and pollination data to provide future climate assessments in Mediterranean basin countries

Konstantia Tolika<sup>1</sup>, Fabrice Requier<sup>2</sup>, Andri Varnava<sup>3</sup>, Menelaos Stavrinides<sup>3</sup>, Giorgio Sperandio<sup>4</sup>, Gianni Gilioli<sup>4</sup>, Fani Hatjina<sup>5</sup>

<sup>1</sup>Department of Meteorology and Climatology, School of Geology, Aristotle University of Thessaloniki, Greece

<sup>2</sup>Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France

<sup>3</sup>Cyprus University of Technology/ CUT- Cyprus

<sup>4</sup>University of Brescia, DICATAM, Via Branze 43, 25123, Brescia - Italy

<sup>5</sup>Department of Apiculture, Inst. Of Animal Science- ELGO 'DIMITRA' -Greece

Climate change is suspected to affect beekeeping and honey bees (*Apis mellifera*) with disturbances in food resource (nectar and pollen) availabilities associated to temperature and lack of precipitation (drought conditions). However, few is known on these climate change effects. In this work we aim at assessing the influence of climate on landscape structure, land use, and on both honey bees and wild pollinators. The assessment was performed using an up-to-date Regional Climate model considering a baseline climatic scenario (1981-2000) and two future scenarios (2041-2060 and 2081-2099). Data used for the assessment were daily precipitation and daily maximum and minimum air temperature. By evaluating the influence of climate on pollination efficiency, biodiversity of bees, prevalence of diseases, honey bee queen performance and honey yields we will produce guidelines to ensure the income of farmers, the resilience of the agroecosystems, and food security in a changing environment. Furthermore, the results will allow the assessment of the role of climate on i) the distribution and availability of feed sources for both *Apis* and non-*Apis* bees, and ii) honey bee health and productivity. Field- and apiary-level data were collected from the following Mediterranean countries: France, Croatia, Slovenia, Greece and Algeria. The results presented are part of the SafeAgroBee project, whose main objective is to develop solutions for adapting and mitigating the effects of climate change and other factors that negatively affect the sustainability and resilience of the Mediterranean agroecosystems.

### OP-196

#### A Data-driven Honey Identification Methodology for Differentiating Honey in the Marketplace

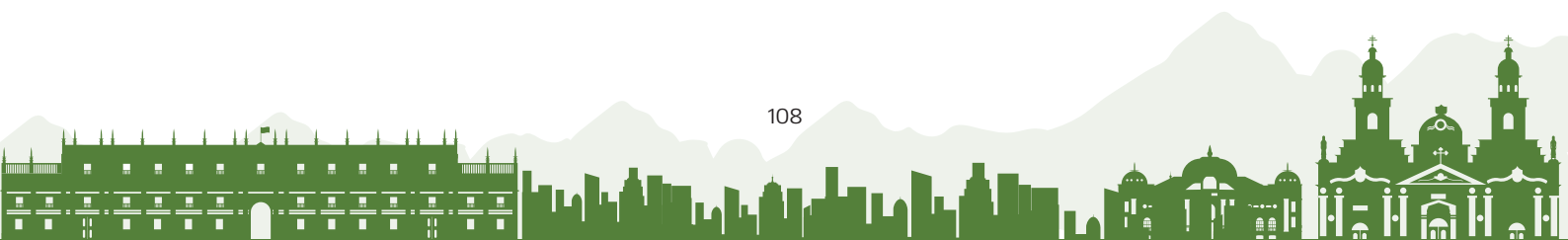
James T. Wilkes, Max Runzel

HiveTracks, Inc. Creston, North Carolina, United States of America

Honey authentication and traceability persist as a global challenge to the beekeeping industry despite years-long efforts to address the problem of economically motivated honey adulteration and willful mislabeling of honey. Lab testing has advanced with NMR testing and additional lab services, but these methods remain costly for small producers with gaps in accuracy, particularly for varietal honeys. Surveillance of honey authenticity in consumer products demonstrates ongoing high levels of fraudulent honey as the fraudsters outpace methods of detection as well as circumvent regulatory policies. The end results are confused honey consumers and an untrustworthy honey market.

HiveTracks creates technology-backed data-driven techniques that use easily accessible data to validate and authenticate honey origin by creating a digital identity for honey at the source of production. When a colony produces honey, the colony location and the time of year provide a definitive basis for identifying the honey. The location dictates a pool of potential nectar producing flowers in the foraging radius of the colony and the date along with the weather and climate conditions determines which nectar sources are blooming and how much honey is produced. Digital documentation of the location of hives, the dates of honey production, and nectar sources blooming at that location is available through a variety of technologies. Photos are tagged with time and location metadata and document the placement, count, configuration, addition of supers, fresh wax/nectar in combs, and harvesting of honey. Hive scales show the beginning, end, and strength of a honey flow. Log files from apiary management software like HiveTracks log apiary management actions. Weather data shows flight hours for nectar gathering. Nectar sources are identified through land use databases, satellite images, and crowd-sourced data like the phenology network and iNaturalist. Additional external authenticity information includes NMR and pollen testing and certified taste tests, all of which can be captured digitally and added to the body of evidence supporting origin and authenticity claims.

We present our honey identification methodology and share working examples that demonstrate the benefits of this framework to the beekeeping community by enabling product differentiation in a beekeeper- and consumer-friendly way.



### OP-197

### Determination of physical and chemical characteristics of bee-pollen for human consumption by near-infrared spectroscopy

Maria Soledad García Paoloni<sup>1</sup>, Federico Vallese<sup>2</sup>, Valeria Springer<sup>2</sup>, Marcelo Fabián Pistonesi<sup>2</sup>, David Douglas De Sousa Fernandes<sup>3</sup>, Paulo Henrique Gonçalves Dias Diniz<sup>4</sup>

<sup>1</sup>Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Hilario Ascasubi

<sup>2</sup>INQUISUR, Departamento de Química, Universidad Nacional del Sur (UNS)-CONICET, Av. Alem 1253, 8000 Bahía Blanca, Argentina

<sup>3</sup>Departamento de Química, CCEN, Universidade Federal da Paraíba, CEP 58051-970, João Pessoa, Paraíba, Brasil

<sup>4</sup>Programa de Pós-Graduação em Química Pura e Aplicada, Universidade Federal do Oeste da Bahia, CEP 47810-059, Barreiras, Bahia, Brasil

Bee-pollen (BP) is considered an excellent nutritional supplement due to its compositional characteristics; actually, the discovery of its biological properties makes it a functional food. In 1990 Argentina incorporated it into its Food Code setting, among others, the moisture and protein content, which take long processing times and contaminating reagents in the latter case. With the aim of improving this situation, near-infrared spectroscopy (NIRS) and Chemometrics was experimented to determinate both parameters and also quantify total polyphenols and flavonoids content to inquire into the possible biological activity. NIRS is a fast, non-polluting and low-cost analytical methodology. The processed samples (62) were collected from 8 apiaries located in the provinces of Buenos Aires (4), La Pampa (1) and Río Negro (3) in two consecutive productive seasons (2013/14 and 2014/15). Spectral data were obtained with a Thermo Scientific Nicolet iS50 FTIR-NIR spectrophotometer. The reference analytical methodologies for moisture and protein content quantification were lyophilization and Kjeldahl. Totals polyphenols and flavonoids content were determined using the individual corbicular loads as the unit of analysis with Folin-Ciocalteu method with gallic acid (GA) standard and the aluminium complex formation with rutin (R) standard in the ethanolic extracts. The lyophilization results ranged from 2.2 to 27.2%; the high variability is due to samples with and without the drying process were considered. Otherwise, the values obtained by the reference methods for the content of polyphenols (0.8-43.4 mg EAG/g), flavonoids (16.4-56.8 mg ER/g) and proteins (14.4 and 26.7%) were also variable, probably due to the different botanical origin. Spectrum data were analysed with chemometric tools with different pre-processing methods (SNV, BO, SGD, MSC). The multivariate calibration models were made with SPXY algorithm, and full-spectrum PLS, SPA-MLR and iSPA-PLS were evaluated for each parameter. The predictive ability of the final models was evaluated in terms of RMSEP, correlation coefficient ( $r$ ), REP and RPD. It can be concluded that the combination of NIRS with chemometrics is a fast, multiparametric, low-cost and non-destructive analytical methodology that could be used for controlling the quality of BP and defining its commercial destination.

### OP-198

### Application of NMR Honey-Profilng method for authenticity control of honey

Léa Heintz, Thomas Spengler, Daniel Santos

Bruker BioSpin GmbH, Rudolf-Plank Strasse 23, 76275 Ettlingen, Germany

Preserving beekeeping is an important economic and ecological task. For instance, 84% of plant species and 76% of food production in Europe depend on pollination by wild and domestic bees.

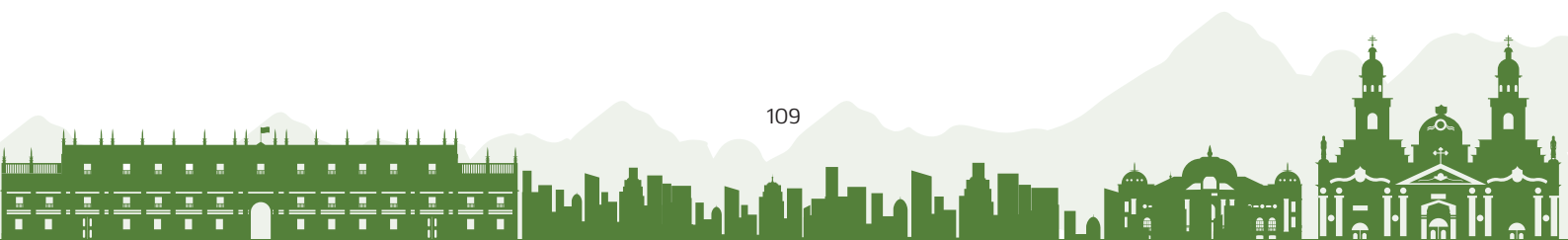
Due to strong competition with adulterated products at low prices, honey fraud threatens the existence of the beekeeping sector and thus the entire food system.

The prevalence on the market of honey adulterated with foreign sugars is unfortunately still at a very high level as assessed through the EU coordinated action called "From the Hives" which found that 46% of honey imported into the EU is suspicious of not complying with the provisions of the Council Directive 2001/110/EC.

Honey is one of the most commonly adulterated foods. The adulteration techniques are diverse and constantly evolving. There is therefore a need for efficient and upgradeable methods that can detect these increasing falsifications.

The application and challenges of authenticity control of honey will be discussed.

The principle of the Honey-Profilng method will be described, as well as the new features of its latest version 3.1.



## Pollination and Bee Flora

### OP-199

#### Optimization of lowbush blueberry pollination and evaluation of honeybees and bumblebees health

Ana María Quiroga Arcila<sup>1</sup>, Pierre Giovenazzo<sup>2</sup>, Valerie Fournier<sup>1</sup>

<sup>1</sup>Laval University, Department of Phytology, Quebec (QC), Canada.

<sup>2</sup>Laval University, Department of Biology, Quebec (QC), Canada.

Canada is the world's leading producer of lowbush blueberries (*Vaccinium angustifolium*) and Quebec is the main producing province. The rental of honeybee hives for pollination accounts for 44.2% of Quebec beekeeper's income and this service represents an expense of approximately \$4 million for lowbush blueberry producers. However, recommendation for optimal honeybee and bumblebee colony density for this crop is unclear. In addition, there are deleterious effects of commercial pollination on pollinators health. The overall objective of this research is to optimize the pollination of lowbush blueberries by honeybees and commercial bumblebees. We tested two honeybee densities (2.5 hives/ha and 5 hives/ha) and one bumblebee density (1.5 multi-hives/ha) and measured the impact on the pollination quality. Nine blueberry fields in the Saguenay-Lac-Saint-Jean region were chosen for our research in the summer of 2022 and 2023. Yields, fruit set, seed set, fruit weight, and fruit quality were monitored along a distance gradient from the colonies (from 25 to 300m). We also measured the foraging activity of both species (number of foragers per species, number of flowers visited per forager, and time spent per flower). The impact of pollination on honeybees and bumblebees' health was evaluated before and after pollination. Variables monitored for honeybees were hive weight, colony strength, various pathogen infestations rates (*Varroa destructor*, *Nosema spp.*, *Ascosphaera apis*, *Melissococcus plutonius*, *Paenibacillus larvae*, viruses: DWV A and B, ABPV, IAPV and BQCV), and the presence of 192 pesticides in honey and bee-bread. Variables monitored for bumblebees were colonies weight, disease development (*Nosema spp.* and *Crithidia spp.*), pesticides presence in pollen, and colony performance (number and weight of individuals and brood per caste). Preliminary results do not show significant differences in fruit yield and fruit set between treatments. The most frequently found pesticides in the fields were the organophosphate insecticide/acaricide coumaphos, and the herbicide hexazinone. Among the visually assessed honeybee diseases, chalk brood (*Ascosphaera apis*) was the most frequently found disease in honeybee colonies, followed by the presence of mosaic brood (undetermined pathology).

### OP-200

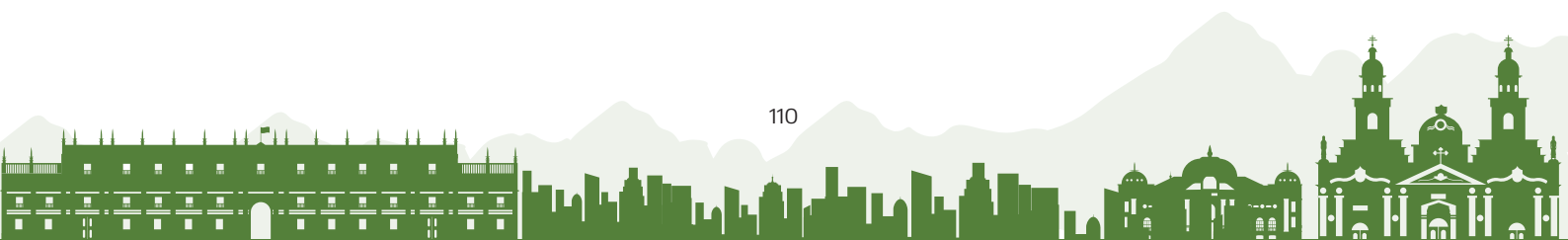
#### Entomofauna associated with botanical species native to Region de Los Rios

Miguel Angel Neira<sup>1</sup>, Leticia Elena Silvestre<sup>1</sup>, Karin Gloria Barrientos<sup>2</sup>, Magaly Riveros<sup>1</sup>, Roberto Carrillo<sup>1</sup>

<sup>1</sup>Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile

<sup>2</sup>Escuela de Graduados, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile.

The Valdivian forest is an eco-region of south-central Chile and some border areas of southwestern Argentina. It is characterized by evergreen forests of multiple strata, in a temperate rainy climate. As representatives of the arboreal stratum are Chilean hazel (*Gevuina avellana*) and ulmo (*Eucryphia cordifolia*); and in the shrub stratum, maqui (*Aristotelia chilensis*) and murta (*Ugni molinae*). Associated with these plants are the visiting insects, which play a very important role in the pollination and reproductive biology of the species. The present study aims to identify the insect visitors of the above mentioned botanical species and to determine their importance in the reproductive biology, establishing factors that influence the pollination of flowers. The field study consisted of measuring the visiting activity (time of permanence TP, frequency of visits FV, among others) of the insects on the flowers and then proceeded to capture them to be identified by taxonomic keys. The results obtained show that Chilean hazel was visited preferentially by *Corynura chloris* and *Apis mellifera*. Midday was the period with the highest VF observed and this was positively correlated with ambient temperature. *A. mellifera* and *Ruizantheda próxima*, presented higher TP during midday. Ulmo was visited more frequently by *A. mellifera*. *Bombus dahlbomii* and *Vespula germanica* only had a strong presence in the early morning hours. *B. dahlbomii* and *Trichophthalma barbarossa* had a lower TP. In maqui, *Policana albopilosa* and *Ruizantheda mutabilis* presented the highest TP. Maqui fruits, larger in size and weight, are achieved in cross-pollination within the first 48 hours of flowering. In murta, *P. albopilosa*, *B. dahlbomii*, *Cadeguala occidentalis* and *Diphaglosa gayi* were the species with the highest PV. The most efficient insects in pollination were of the order Hymenoptera, since they presented the greatest number of grains in their bodies. In general terms, the flower-visiting insects of the botanical species are native, and therefore play a fundamental role in their pollination.





### OP-201 Bees under cover

Carolyn Anne Sonter<sup>1</sup>, Susan Caroline Wilson<sup>1</sup>, Blake Dawson<sup>1</sup>, Jeremy Jones<sup>1</sup>, Jared Reid<sup>1</sup>, Charlie Bailey<sup>1</sup>, Steven Fuller<sup>2</sup>, Matthew Tighe<sup>1</sup>, Romina Rader<sup>1</sup>

<sup>1</sup>Environmental and Rural Science, University of New England, Armidale, NSW, Australia

<sup>2</sup>Bee Services, Grafton, NSW, Australia

Protective crop covers are used extensively to protect high-value crops from bird and hail damage however, these structures may present challenges for honey bees (*Apis mellifera* L.). Although protective covers safeguard crops from weather and animal damage, the altered temperature, light and visibility conditions can impact honey bee foraging behaviour, access to resources and honey bee colony viability. Some studies have indicated that protective covers result in a decline in colony size and resource storage, however, few replicated field trials exist. To determine the impact of protective cropping structures on honey bee brood production and resource storage, honey bee colonies were placed inside and outside of protective covers on four blueberry farms of northern New South Wales, Australia. Each fortnight over a 12 week period, we measured changes in brood production, pollen and honey storage, pollen collection, hive weight and internal hive temperature. Brood production, pollen and honey storage, and hive weight all decreased within four weeks of placement under net cover. Although these four metrics gradually increased in all hives during the remainder of the 12-week monitoring period, hives located under protective netting had relatively smaller gains in brood production, stored honey and pollen when compared to hives located outside the protective covering. Hives under protective netting that were in the shade and/or whose entrances were facing south were most impacted by the end of the monitoring period. Our findings highlight the need for protected crop management strategies to consider pollinator health as well as crop pollination needs so that both bee health and pollination services are maintained in these systems.

### OP-202 Managed Native Bees (*Scaptotrigona Aff. Postica*) and Wild Pollinators Impact on Açai Palm (*Euterpe Oleracea Mart.*) Yield In Eastern Brazilian Amazon

Márcia Motta Maués<sup>1</sup>, Alistair Campbell<sup>2</sup>, Felipe Deodato Da Silva e Silva<sup>3</sup>, Kamila Leão<sup>4</sup>, Luisa Carvalheiro<sup>5</sup>, Eduardo Freitas Moreira<sup>6</sup>, Frédéric Mertens<sup>7</sup>, Maria Luiza Konrad<sup>8</sup>, Cristiano Menezes<sup>9</sup>

<sup>1</sup>Laboratório de Entomologia, Embrapa Amazônia Oriental, Belém, Brazil

<sup>2</sup>Natural England, Lancaster, UK

<sup>3</sup>Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso, Barra do Garças, Brazil

<sup>4</sup>Serviço Nacional de Aprendizagem Rural - SENAR/PA - Belém, Brazil

<sup>5</sup>Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, Brazil

<sup>6</sup>INCT IN-TREE, Universidade Federal da Bahia, Salvador, Brazil

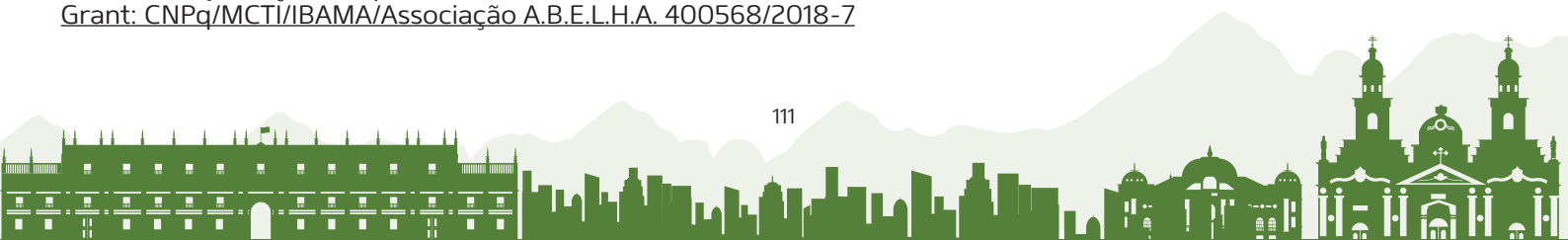
<sup>7</sup>Centro de Desenvolvimento Sustentável, Universidade de Brasília, Brasília, Brazil

<sup>8</sup>Universidade Federal do Tocantins, Palmas, Brazil

<sup>9</sup>Embrapa Meio Ambiente, Jaguariúna, Brazil

Agricultural expansion is a major driver of habitat loss, which triggers biodiversity decline, including wild pollinators, and affects crop production. 76% of world crops are dependent on biotic pollination, therefore Integrated Crop Pollination (ICP), the arrangement of managed and wild bees combined with farm practices that support wild pollinators (e.g. habitat management), could help reverse negative impacts of pollinators deficit in tropical crops, while maximising benefits to producers. In the Amazon River delta, the Açai palm (*Euterpe oleracea* Mart. Arecaceae) is unmatched among native forest plants in its cultural, social and economic importance to the region. Its thick juice ("vinho do açai") is an important staple food in both rural and urban populations. We applied the ICP approach to açai palm production in the eastern Brazilian Amazon and evaluated the effects of a native managed stingless bee, *Scaptotrigona postica*, and landscape-level forest conservation on yield and socioeconomic outcomes, on 18 açai palm plantations in northeast Pará state, Brazil. We found that managed stingless bees and forest cover enhanced flower visitor abundance on açai palm inflorescences, but visitor abundance increases attributed to managed bees were associated with shifts in flower visitor evenness and diversity, due to reduced visitation of wild bees close to managed colonies. Fruit production on inflorescences was positively related to bee abundance and bee diversity. Consequently, overall pollination efficiency was lower in plantations dominated by managed bees, especially when native forest cover in the surroundings was low. At the hectare scale, managed bees and landscape-level forest conservation had complementary effects on fruit yields, but additional costs of bee colonies mean profits were largely explained by surrounding forest cover. We concluded that managed bees have great potential to boost açai fruit yields, but the increased environmental and socioeconomic risks associated with this activity indicates that growers should prioritise forest conservation and habitat restoration to safeguard natural pollination ecosystem service and improve the overall sustainability of açai fruit production in the eastern Brazilian Amazon.

Grant: CNPq/MCTI/IBAMA/Associação A.B.E.L.H.A. 400568/2018-7





### OP-203

#### Management of floral resources for development of beekeeping in India

Lakshmi Rao Kavuri

Central Bee Research and Training Institute, Pune, India

India is endowed with a variety of vegetation types ranging from alpine and temperate forests to tropical deciduous scrub jungles, corresponding to the climate, soil and other factors. The country has about 73.62 million hectares under forests and an area of 196.43 million hectares is under cultivation. An important constraint for implementing the beekeeping is the lack of information on bee forage resources. Survey has been conducted and observations were made on visits of bee foragers. During the periodic visits general study of the local vegetation and survey was made to evaluate the floral resources of "Apis" species were recorded. 500 honey samples and pollen loads/stores were collected from beehives throughout the country and melissopalynological investigations carried for confirmation. A total of 674 plant species belonging to 528 genera in 86 families were recorded as sources of pollen or nectar or both. There were 237 natural, 343 cultivated and 94 weed or wild species. 340 monofloral honeys belonging to 51 plant species of which 31 new records of nectar sources in India is reported. The data collected during the survey is formulated as floral calendars for 550 districts of India state wise. India is endowed with a rich variety of floral resources that provide pollen and nectar. Bees can get forage for almost 9 months in a year. Proper utilization of this rich potential will improve honey production in India. Migration of bee colonies to locations where bee forage is available is an integral part of beekeeping. Data generated in the present investigations on floral sources in different phytogeographic regions was utilized to formulate some migratory routes and schedules for adoption by beekeepers. The knowledge gained can have great utility in evolving appropriate management schedules for migratory beekeeping or for production of 'organic' honeys and thus in optimal utilization of the floral resources for improving productivity and quality of honeys in India. There is potential to produce monofloral honeys from over 50 plant species if bee hives are migrated to locations where the species abound.

### OP-204

#### Consequences of the high abundance of *Bombus terrestris* on the pollination of *Vicia faba*

Cecilia Smith Ramirez<sup>1</sup>, Adriana Estefanía Rendón Funes<sup>2</sup>, Rodrigo Barahona Segovia<sup>3</sup>, Wladimir Moya<sup>3</sup>

<sup>1</sup>Universidad de Los Lagos, Institute of Ecology and Biodiversity (IEB), Universidad Austral de Chile

<sup>2</sup>Institute of Ecology and Biodiversity (IEB), Museo de Historia Natural Alcide d'Orbigny

<sup>3</sup>Universidad de Los Lagos

One of the main visitors to *Fabia* bean crops (*Vicia faba*) in South America is the invasive bumblebee species *Bombus terrestris*. This is particularly true in Chile, where *B. terrestris* was first introduced in 1997 and is now common over much of the country. In this study, we evaluated the activity of the principal pollinators of *V. faba* over two cropping seasons by assessing their visitation rates while distinguishing between legitimate visits, likely to lead to pollination, and nectar robbery. We then determined the net contribution of insect visitation on pod and seed set. We recorded seven species of floral visitors. Most visits (legitimate visits and robbery) were from the honeybee (*Apis mellifera*), with *B. terrestris* being the next most common visitor. In the case of *B. terrestris*, 87.19% of visits were nectar robbery. On average, the same flower perforation was visited 23 times by *B. terrestris* during the flower's lifespan. In general, the frequency of legitimate visits varied with pollinator identity and year. For *B. terrestris*, each flower received an average of 0.95 legitimate visits during its entire lifespan. The time spent by *B. terrestris* visiting flowers for both nectar robbery or pollen collection decreased after the first day of flowering suggesting resource depletion. The number of pods, total seed number, and seed weight were lower where self rather than open pollination. This suggests that open pollination increased reproductive success. We conclude that *B. terrestris* was likely to contribute relatively little to pollination while at the same time depleting floral resources throughout the flowers' lifespan. *Bombus terrestris* is likely to make *V. faba* flowers less attractive to other more effective pollinators. As a result, this may potentially drive interspecific competition for pollen and nectar within the crop.



### OP-203

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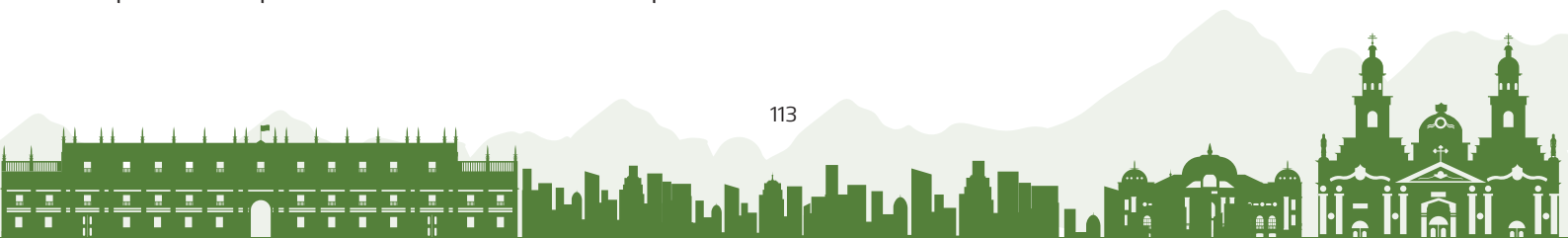
Cecilia Smith Ramirez<sup>1</sup>, Adriana Estefanía Rendón Funes<sup>2</sup>, Rodrigo Barahona Segovia<sup>3</sup>, Wladimir Moya<sup>3</sup>

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### OP-205

#### Potential use of stingless bees hives for blueberry crop pollination

Patrícia Nunes-Silva<sup>1</sup>, Murillo Jesus<sup>2</sup>, Manoela Lencino Sant Anna<sup>1</sup>, Betina Blochtein<sup>2</sup>

<sup>1</sup>Polytechnic School, University of the Sinos River Valley, São Leopoldo, Brazil

<sup>2</sup>School of Health and Life Sciences, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Blueberry cultivation in Brazil has grown over the last decades, but knowledge on its pollination is still limited in the country. Among potential managed pollinators, species that buzz pollinate are of particular interest, e.g. stingless bees of the genus *Melipona*, because blueberry flowers have poricidal anthers. We evaluated *Melipona quadrifasciata* and *Melipona torrida* as blueberry crop pollinators in a 415m<sup>2</sup> blueberry (*Vaccinium ashei* Reade) orchard of a farm located in southern Brazil (30°07'50.1"S 51°25'33.3"W), during the flowering period (September and October 2019). Four hives of each bee species were introduced on the side the orchard, 3m apart each other. Pollen was collected from the foragers' corbicula four times a day (9am, 11am, 1pm and 3pm), during five minutes for five days. It was stored in 70% alcohol, separated by forager, hive, sampling time and day, and bee species. At the laboratory, under a microscope, 1200 pollen grains were counted per forager, registering how many were blueberry. Furthermore, we counted the number of visits per visitor species three times a day (11am, 1pm and 3pm) in two 20m transects during one min per plant of each transect (8 to 12 plants) for 6 days. Only one of the sixteen pollen foragers of *M. quadrifasciata* sampled carried blueberry pollen and had only two pollen grains in its pollen load. Although 134 *M. torrida* foragers were sampled, only 31 were carrying blueberry pollen, with a mean number of 1.2 grains per forager (standard deviation = 3.3; maximum = 28). The mean percentage of blueberry pollen present at *M. torrida* pollen loads was 0.15%. During the survey of the floral visitors, only 20 (0.2%) visits of *M. quadrifasciata* and 28 (0.2%) of *M. torrida* were recorded, indicating that they were not frequent visitors when compared to honeybees (74%) and the stingless bee *Trigona spinipes* (17%). These results indicate that blueberry flowers were not a pollen source for *M. quadrifasciata* and *M. torrida* and, although they visit them, the visitation frequency is low. Unless management techniques are developed to increase their visitation on blueberry flowers, their potential in blueberry managed pollination is not promising.

### OP-206

#### Floral resource specialization at the individual-level in a generalist population-level of a solitary bee species

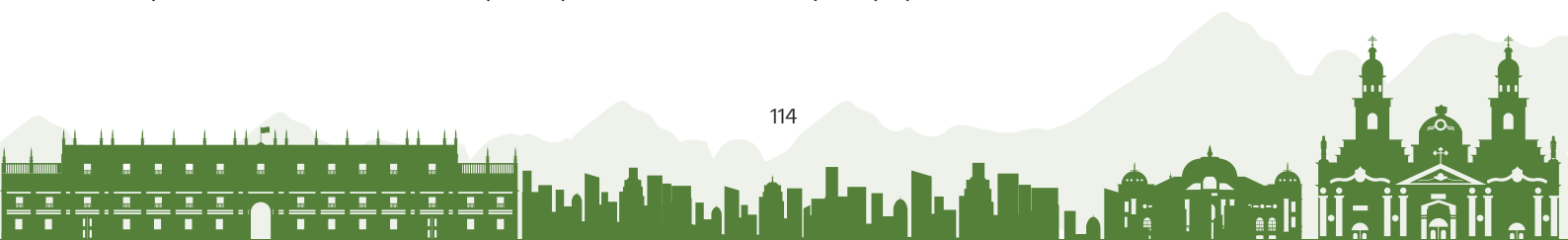
Diego Moure-Oliveira<sup>1</sup>, Léo Correia Rocha-Filho<sup>2</sup>, Carlos Alberto Garófalo<sup>3</sup>

<sup>1</sup>AgroBee, Ribeirão Preto, SP, Brazil

<sup>2</sup>University of Uberlândia, Uberlândia, MG, Brazil

<sup>3</sup>University of São Paulo, Ribeirão Preto, SP, Brazil

The plant-pollinator interactions are complex and understanding these interactions is fundamental in the strategic planning of conservation and applied pollination projects. Ecological differences at individual and populational level on floral source interactions can be observed, where some generalist populations are not necessarily composed of generalist individuals but may be composed of individuals that differ from each other in terms of exploiting floral sources. Bees are insects that collect distinct floral resources to different functions. While pollen is the protein food source and used to feed their offspring, the nectar is the energy source used to feed the offspring and to feed the forager bee; finally, some bee species collect floral oil to build their nests. The aim of this study was to investigate the relationship between the solitary oil-collecting bee *Centris analis* (Apidae, Centridini) and its floral resource sources. Nineteen females were accomplished during the reproductive period, between October and December 2015, at a nesting site in Ribeirão Preto, SP, Brazil, totalizing 406 hours. Using the trap nest methodology, the residual pollen present in the brood cells was submitted to the acetolysis process, and the pollen grains were counted, identified, and separated in pollen sources, nectar sources and floral oil sources. Ecological analyzes of interaction networks were performed in the DIETA1 software. Three plant species were recognized as pollen source, twenty-nine as nectar source, and only one as floral oil source. Although this species can be considered generalist and polylectic according the populational-level, the individual diet analysis showed distinct patterns depending on the resource explored. In the individual-level, while a high individual specialization in the nectar foraging was observed ( $E = 0.89$ ,  $p < 0.001$ ), a lower individual specialization in the pollen foraging ( $E = 0.65$ ,  $p < 0.001$ ), and no diversity in the individual floral oil foraging, where only one plant species was visited, the Indian cherry tree (*Malpighia emarginata*, Malpighiaceae). The generalist potential - at least for nectar collection - demonstrates the foraging potential of this solitary oil-collecting bee species and help to better understand the plant-pollinator relationship, at populational and individual-level.



### OP-207

#### How self-pollinating crops can benefit from bee pollination: the case of the stingless bee *Trigona fuscipennis* and cowpea (*Vigna unguiculata*)

Breno M. Freitas, Vitória Inna Mary De Sousa Muniz, Pedro De Assis De Oliveira, Larysson Feitosa Dos Santos, Kiara Leite Santos Ferreira, Letícia Ferreira Paiva

Departamento de Zootecnia, Universidade Federal do Ceará, Fortaleza-CE, Brasil

Flowers of various crops, such as soybeans, beans and cowpea, are capable of self-pollination and produce viable crops. However, self-pollination does not always provide the best results. We investigated how cowpea (*Vigna unguiculata* (L.) Walp.), an important species for food security in many developing countries, can benefit from biotic pollination, specially by the stingless bee *Trigona fuscipennis* (Friese). The experiment was carried out from May to June 2022 in the Federal University of Ceará, Brazil, in a 94.5m<sup>2</sup> area cultivated with the Guariba variety, evaluating seed-setting rate (%), number of seeds set per pod, seed weight (g) and days to harvest as a result of four treatments: open pollination (OP), spontaneous self-pollination (SS), manual cross-pollination (MCP) and one *Trigona fuscipennis* visit (TF). There were differences ( $p < 0.01$ ) in setting rate, with MCP (58.15%) and SS (65.94%) having set significantly fewer seeds than TF (85.87%). TF, however, did not differ from OP (78.35%), which in turn did not differ from SS and MCP. The number of seeds per pod also differed between treatments ( $p < 0.01$ ), with OP ( $10.45 \pm 4.65$ ) and TF ( $10.12 \pm 3.85$ ) differing from MCP ( $6.89 \pm 4.13$ ). MCP, in turn, did not differ from SS ( $8.23 \pm 4.25$ ) and the latter also did not differ from OP and TF. Regarding seed weight, OP ( $1.85 \pm 0.89$ g) and TF ( $1.62 \pm 0.64$ g) also differed ( $p < 0.01$ ) from MCP ( $0.81 \pm 0.60$ g), but MCP did not differ from SS ( $1.36 \pm 0.78$ g) and SS also did not differ from TF. Seeds from OP ( $16.75 \pm 4.48$  days) and TF ( $17.00 \pm 3.32$  days) matured significantly ( $p < 0.01$ ) faster than those from SS ( $20.34 \pm 5.03$  days) and MCP ( $20.72 \pm 3.66$  days). We concluded that the biotic pollination as observed in OP produces a higher rate of seed setting and heavier seeds that mature earlier than when the plant depends only on spontaneous self-pollination. Also, a visit by *T. fuscipennis* to the flower already produces similar results to the OP, perhaps because they were the vast majority of floral visitors. Manual cross-pollination showed the worst performance, suggesting that it is not favorable to the crop or errors in handling the flowers. Other self-pollinating crops may present similar behavior and deserve investigation.

### OP-208

#### Supplemental pollination and soybean yield

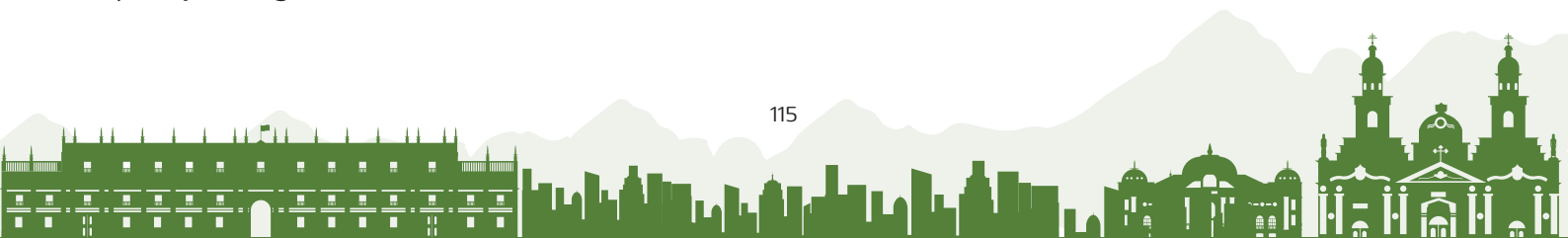
Decio Luiz Gazzoni<sup>1</sup>, Clara Beatriz Hoffmann Campo<sup>1</sup>, Guilherme Julião Zocolo<sup>1</sup>, João Vitor Ganen Rillo Paz Barateiro<sup>2</sup>, Heber Luiz Pereira<sup>3</sup>

<sup>1</sup>Researcher, Embrapa Soybean, Brazil

<sup>2</sup>Doctorate student, Universidade Federal de Lavras and Embrapa Soybean

<sup>3</sup>Post-doctorate student, Universidade Estadual de Maringá and Embrapa Soybean

Brazil is the leading global soybean producer, with ca. 42 million hectares grown last season. In recent years, beekeepers have shown a growing interest in migrating their hives close to soybean fields to allow honeybees, *Apis mellifera* L. 1758 (Hymenoptera – Apidae), to forage in their flowers. We assessed the effect of honeybee pollination on the soybean yield through two experiments set up in Londrina, Brazil. The soybeans yield and its components, to whose flowers were visited by honeybees, were evaluated compared to those not. The first experiment, concerning a high soybean yield potential, was repeated for three consecutive years during the 2017 to 2020 soybean growing seasons. Treatments consisted of open plots with freely visiting honeybees, caged plots with a honeybee hive inside, and caged plots with no honeybees or other pollinators. The second, with the same treatments, was set up under conditions of low soybean yield, repeated in the 2016/17 and 2019/2020 seasons. Monitoring honeybees visiting soybean flowers indicated consistent visits in open and caged plots with a beehive and the highest number of honeybees visiting soybeans at 11 am. The average yield of the 1st experiment in three years-trials revealed an increase when honey bees had access to flowers, reaching 5,565 kg/ha for caged plots with honey bees and 5,201 for open plots, compared to caged ones without honey bee hives, which yielded 4,926 kg/ha. The yield increase was mainly associated with the higher occurrence of 3 and 4-seeded pods, a higher number of seeds/pod and seed weight, as well as a smaller number of pods with zero or one seed in the plots with the presence of honeybees, compared to the caged plots without beehives. For the 2nd experiment, results indicated no yield differences due to the pollination of soybean flowers by the honeybees, compared to the treatment with their absence. We concluded that soybean yield increment by the supplemental pollination due to honeybees is associated with a high-yield environment and adequately managed farms.



### OP-209

#### Floral visitors of *Tradescantia pallida*

Isac De Macedo Da Penha, Alessandra Fernandes Gonçalves Benites, Vagner Alencar Arnaut Toledo

Department of Animal Science, State University of Maringá, Brazil

Most species of trees in tropical forests are pollinated by insects, especially bees, which are the main pollinators of plants. *Tradescantia pallida* is a plant species widely used as an ornamental plant due to its ease of cultivation and high resistance to climate and environmental factors. Observing the behavior of pollinators is difficult due to the speed of their movements in flowers, as well as many of them working at inconvenient times of the day or in hard-to-reach locations. The present study aimed to observe the flowers of *T. pallida*, located at the State University of Maringá, Maringá-Paraná-Brazil in July 2022. Its flowers have no nectaries and open around 6:30 am in the morning, with a one-day anthesis and closing occurring around noon. During the observations, two floral visitor species were identified: Africanized *Apis mellifera* and *Tetragonisca angustula*. It was found that the plant species provides only pollen as a resource for bees. It was observed that Africanized *A. mellifera* bees are the effective floral visitors of *T. pallida* due to their ability to collect pollen from up to two stamens at the same time. In addition, these bees collect more pollen due to the larger size of their corbiculae and greater number of individuals in the colony. On the other hand, *T. angustula* bees are able to collect pollen from only one stamen, but in a longer time than Africanized *A. mellifera*. From 9 am, *T. angustula* bees began to visit the flowers, with a greater number of bees observed from 10 am to 11 am. Despite a higher flow of Africanized *A. mellifera* bees, there was no competition between the observed species. For this plant species, no nectar guides or nectaries were observed in the flowers, which justifies the collection of only one resource by bees. This study provides important information on the behavior of pollinators in a common ornamental plant species, which can help in understanding the ecology and evolution of these organisms.

### OP-210

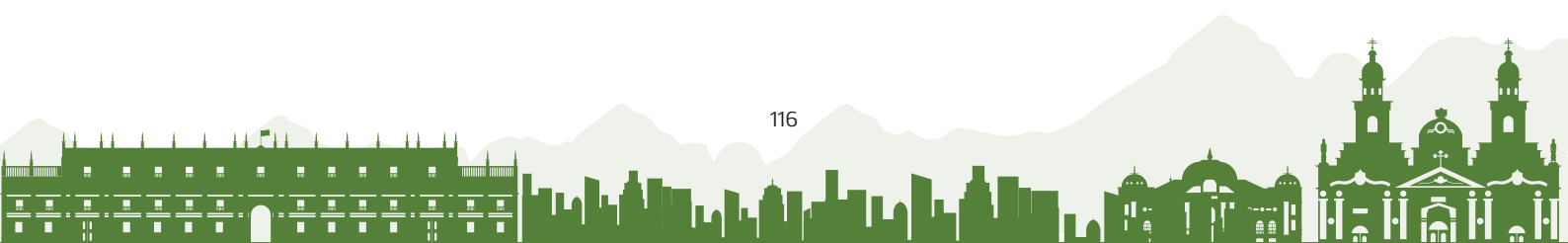
#### Typical Multifloral honeys from the Pampas Austral District and Espinal Caldén District (Argentina)

Gisela Grandinetti<sup>1</sup>, Elian Tourn<sup>2</sup>, Liliana María GALLEZ<sup>1</sup>

<sup>1</sup>Laboratorio de Estudios Apícolas (LabEA) UNS-CIC, Bahía Blanca, Argentina.

<sup>2</sup>Laboratorio de Estudios Apícolas (LabEA) UNS-CIC; Cooperativa de Trabajo Apícola Pampero Limitada, Bahía Blanca, Argentina.

The characteristics of raw honey depend primarily on the plant species visited by bees. The southern Pampas (Austral District of the Pampas phytogeographic province), which is a vast prairie area where the plant communities are linked to edaphoclimatic conditions and agricultural practices. The Ventania mountains divide this region into a sub-humid/humid zone and a semiarid zone. The adjacent phytogeographic region, called Monte by local beekeepers, is situated in the Caldén District of the Province of Espinal, where native nectariferous shrubs and trees are dominant. The CAP beekeeper's association employs a traceability system and apiary geolocation and has partnered with LabEA UNS-CIC. CAP offers four multifloral honey types: Pradera húmeda (PH), Pradera Seca (PS), Cordón Serrano (CS), and Monte Nativo (MN). These honeys are produced in the sub humid/humid prairies, semiarid prairies, Ventania mountains, and Caldén District, respectively. Prior studies conducted by LabEA have verified the origin of these honeys by using pollen, sensory, and physico-chemical analyses. The combination of *Eucalyptus* sp., *Diplotaxis tenuifolia* and *Centaurea* sp. pollen types can be found throughout the region in varying proportions. PH honeys are extra light amber/light amber (34-85 mm Pfund) and often have greenish hues due to sunflower nectar. The aroma is described as having green vegetable notes and coumarin (from *Melilotus* sp.). PS honeys have a firm crystallization and <50mm Pfund, with subtle warm and dried vegetable notes related to *Centaurea solstitialis* and floral and pungent notes associated with *D. tenuifolia*. CS honey's origin is mainly confirmed by the combination of pollen grains from many spontaneous plants, such as *Echium* sp., *Baccharis* sp., *Mimosa rocae*, among others. The colour of CS honey may range from white to light amber, and the aroma is characterized by floral/fresh fruit, vegetal, and warm notes. MN honeys have pollen combinations from *Condalia microphylla*, *Neltuma* sp. (= *Prosopis* sp.), *Larrea divaricata*, *Schinus* sp., *Discaria americana*, etc. The colour of MN honey ranges from 50-140mm Pfund, and the odour intensity is medium-high, with warm and woody aromas and animal notes typical of the Rhamnaceae family. These regional honey studies allow beekeepers to offer unique products.





### OP-211

#### What are the frequently interacting species in plant-pollinator networks?

Ehsan Rahimi<sup>1</sup>, Chuleui Jung<sup>2</sup>

<sup>1</sup>Agricultural Science and Technology Institute, Andong National University, Andong 36729, Republic of Korea

<sup>2</sup>Department of Plant Medical, Andong National University, Korea

Pollinators play a critical role in maintaining biodiversity and ensuring the reproduction of many plant species. By studying plant-pollinator networks, researchers can develop targeted conservation and restoration strategies to ensure the long-term sustainability of both plant and pollinator communities. In this context, the objective of this research is to identify the most frequently interacting species in plant-pollinator networks and to analyze available data related to plant-pollinator networks on a global scale at different taxonomic levels. Using data from approximately 37000 interactions between plants and pollinators, we identified highly visited pollinators and host plants across 205 networks worldwide. Through taxonomic analysis, we found that there are 1979 and 1249 genera of pollinators and plants respectively among them that bees and flies genres are among the most visiting pollinators. Also, Certain plant families and orders were found to be more attractive to pollinators, which could be useful in designing more effective strategies for crop pollination. Certain plant families such as Asteraceae, Fabaceae, and Rosaceae may be particularly important for sustaining pollinator populations, and this could have implications for conservation efforts or agricultural practices. The pollinators and plants investigated were classified into 25 and 48 orders, respectively. pollinator orders, such as Hymenoptera, and Diptera, and plant, orders such as Asterales, and Lamiales were recognized as the most frequently interacting orders. One significant outcome of this study is that taxonomic studies can provide valuable insights into the interactions between plants and pollinators, by identifying higher-level taxonomic categories that are important for supporting these interactions. Overall, the study provides important insights into the patterns of interaction between pollinators and plants, which could help to inform more effective strategies for crop pollination and the conservation of pollinator populations.

### OP-212

#### microscopic investigation of honey to develop melliferous floral diversity of agroecological zone of pakistan

Mehwish Noor

Department of Environmental sciences. Fatima Jinnah Women university.The Mall.Rawalpindi

Honey bee keeping provides an entrepreneurship to the rural family to keep them financially stable and nurture the sustainability as bees play vital role in biodiversity, pollination, revenue generation through honey production and poverty reduction. In the present study survey of melliferous flora was carried out to develop inventory of floral specific sites. Two hundred and five (205) plant species are identified belonging to sixty seven families. Including wide range of wild, cultivated, ornamental, exotic, crop, anemophilous flora like *Justicia adhatoda*, *Acacia* sp., *Ziziphus jujuba*, *Taraxicum officinalis*, *Artemisia absinthium*, *Pinus* sp, *Pennisetum* sp., *Pyrus*, *Prunus*, *Eryobotria*, *Citrus*, *Eucalyptus* sp. or *Parthenium hysterophorus* indicating the significance of those plants in bee industry. Alternatively bee flora identification was carried out through mellissopalynology. For this purpose sixty four honey samples were collected during field survey. Studied sample expressed diverse spectrum of sixty different pollen grain of thirty seven plant families. Fabaceae, Asteraceae, Poaceae and Myrtaceae family's pollen were most abundant. Sixty one percent of studied honeys were unifloral and thirty nine percent were multifloral. *Trifolium repens* L., *Acacia modesta* Wall, *Eucalyptus cammoldulensis* Dehnh, *Brassica campestris* L., *Citrus sinensis* L., pollen were dominant or frequent. The Absolute Pollen Count of studied honey suggests: 73% samples belong to Group I, 18.7% to Group II and 8.7% to Group III of Maurizio's Categories. Poor value of Honey dew element i.e. 0.02-0.38 indicated good quality of honey. Identified rich bee flora of Pakistan highlights the training of the farmers and the locals to promote biodiversity entrepreneurship in honey industry.



## Pollination and Bee Flora

### OP-213

#### Preserving and increasing biodiversity using beekeeping in commercial & private settings

Paula Carnell

Paula Carnell Limited

With over 22,000 species of bees across the Earth, pollination emphasis has been concentrated on the honey bee who is not the most effective of our pollinators. Solitary and bumble bee species have evolved over millennia to pollinate specific species of plants and their families. Due to human developments in agriculture and our impact on the environment, many of these species have been lost or are under threat. Reliance on the honey bee to pollinate our crops is now at an unsustainable level and so a wider understanding of the problem, and solutions needs to be shared. Working on a smaller local scale, I have found that education for farmers and landowners is vital, and welcomed when they learn the impact biodiversity can have on their profits, as well as developing a strong media story that can increase brand loyalty. With a 'bee program' I begin with a thorough 'bee audit' across the landowners estate and farmland, locating wild and managed honey bee colonies, as well as the species of solitary and bumble bee species currently visiting their land. Recommendations can then be made on increasing specific forage for the most essential pollinators for their specific crops. For example, the red mason bee which is a more effective pollinator of Apples in the South West of England, (100-200 times) requires 2 year old dried plant stems to nest in, creating suitable habitat sufficient to attract and maintain local native bee species decreases the reliance on honey bees. On one commercial cyder orchard in Somerset, we increased biodiversity from 8 species of bees to over 26 in 5 years. Increased yields and creating a media story about their bee program has encouraged more investment in chemical free agriculture and biodiversity in planting and land management. By using only native honey bees from the area, no damaging effect on the other species of bees occurs, leaving the honey bees to concentrate on nectar rich plants to create honey, minimising competition amongst species. 'Bee Safaris' for visitors and clients increases awareness and develops into a movement of change.

### OP-214

#### Native bees pollination impact on the regeneration of tropical dry broadleaf forests in Chaparrí (North coast of Peru)

Fernando Serván López<sup>1</sup>, Carlos Wong Un<sup>1</sup>, Ricardo Rabanal Rivera<sup>2</sup>, Heinz Plenge<sup>2</sup>

<sup>1</sup>VISION 2050

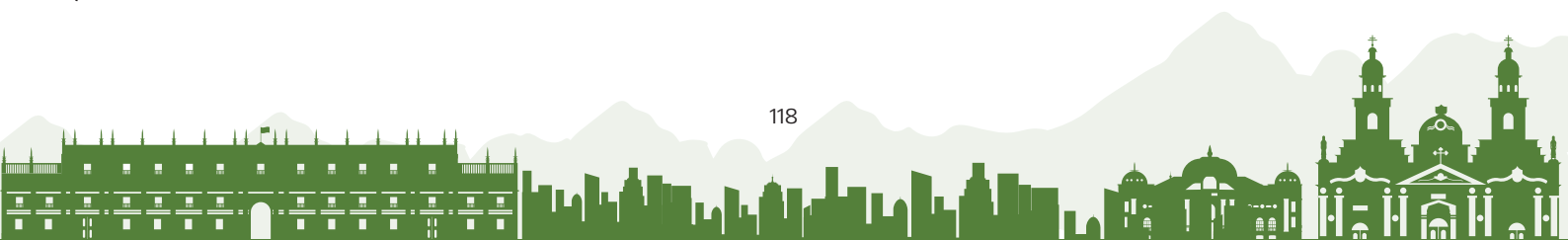
<sup>2</sup>Asociación Tu Tierra

The "Bear and Honey Route" project is an initiative of the associations "VISION 2050" and "Tu Tierra" in Peru, and Bioparc de Doué-la-Fontaine, France. It seeks to ensure the conservation of biodiversity and the regeneration of the dry forest through the development of meliponiculture as a productive and sustainable activity for local communities in the project area, which is the protected natural area of Chaparrí, Chongoyape district, Chiclayo, Lambayeque, Peru.

As part of the project, the aim of this study was to understand the interactions between native bees and plant species in the tropical dry broadleaf forests in Chaparrí (North coast of Peru) with the goal of improving plant-pollinator interactions for the regeneration of the tropical dry broadleaf forests in the North coast of Peru.

Samples of honey from two native bees (*Melipona eburnea* and *Plebeia* sp.) were collected using sterile syringes and sterilized 350 ml dry bottles. Seven samplings were performed for each species. Melissopalynology analyses were carried out, detecting the presence of pollen grains belonging to the Anacardiaceae family with 90% of the *Loxoterigium huasango* "hualtaco" species (for *Plebeia* sp.) and of the Fabaceae/Mimosoidae family with 65% of *Acacia macracantha* "faique" species (for *Melipona eburnea*). The analyses were carried out in Germany (Eurofins laboratory) and Lima (CERPER laboratory).

These preliminary results confirm the effectiveness of native bees, particularly *Plebeia* sp., in pollinating plants that are in the "critical danger" category such as "hualtaco", and of *Melipona eburnea* in pollinating *Acacia macracantha*, two of the main plant species in the protected natural area. Further studies will be conducted to confirm the importance of preserving and restoring plant-pollinator interactions, involving *Plebeia* sp., *Melipona eburnea*, and other native bee species, which are critical for maintaining healthy ecosystems and ensuring the survival of both plant and animal species.



### OP-215

## The EU Pollinator Hub: Collective approach to understanding pollinator trends and threats and providing data analytical service to society

Noa Simon Delso<sup>1</sup>, Bastien Crespy<sup>1</sup>, Gregor Susanj<sup>2</sup>, Gilles San Martin<sup>3</sup>, Michael Rubinigg<sup>4</sup>

<sup>1</sup>BeeLife European Beekeeping Coordination

<sup>2</sup>Zdruzba IP d.o.o.

<sup>3</sup>Centre de Recherches Agronomiques de Wallonie

<sup>4</sup>Visualife

It is well established that insect pollinators' numbers and diversity have severely decreased over recent years in Europe and worldwide. Beekeepers have experienced increased colony losses, and their observations are key to understanding and following up on insect decline and production trends, which are worrying as many sectors and actors depend on pollinators and pollination for their activity. Consequently, bees and other insect pollinators are taking increasing relevance in the public debate, and growing efforts have been made over the years to understand these trends and the factors that may affect them. Related data have been/is produced by different institutions and actors, with various purposes and in multiple formats, making it impossible to obtain a clear picture of the situation and, more importantly, the possibilities to reverse the trends. In Europe, the stakeholder group EU Bee Partnership has created an innovative technology in data connectivity and dissemination applied to the environment: The PollinatorHub.eu). By sharing or including our data and observations in the Hub, each of its users contributes to generating knowledge on the topic that everybody can use while keeping confidentiality and copyright. Thanks to its horizontal approach, the PollinatorHub can provide advice on field data collection, curation and communication, do the data curation, standardisation or analytics for the users, and provide meaningful and informative data visualisation options, so its users get the best out of their data. Beekeepers collecting data but not knowing how to analyse it can profit from the analytical features of the Hub. Researchers in bees, beekeeping and pollination can use the Hub to host the data as a data repository that will centralise the pollinator-related data. The PollinatorHub platform aims to transform the existing segregated data into a reference tool for those seeking information or whose activity depends on pollinators and pollination and who need to adapt their activities to manage the drivers determining their fate.

### OP-216

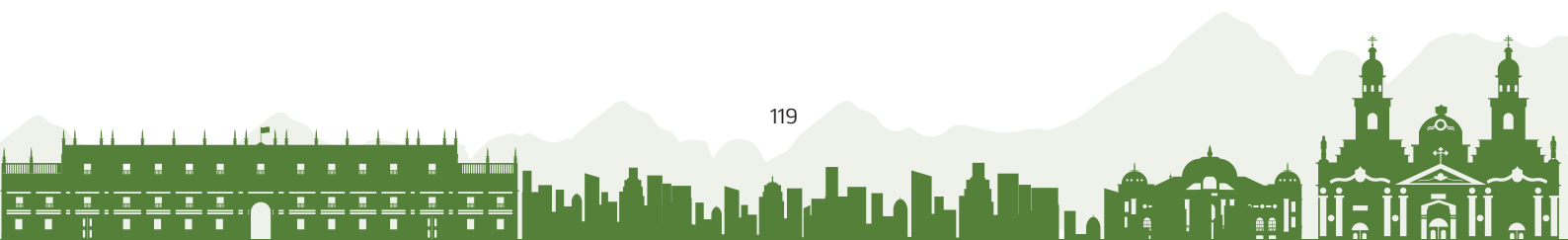
## Comparison of flower traits and visiting pollinator insects of *Prunus davidiana* and *Prunus salicina*

Kwanhui Lee<sup>1</sup>, Sukjun Sun<sup>1</sup>, Chuleui Jung<sup>1</sup>, Chuleui Jung<sup>2</sup>

<sup>1</sup>Department of Plant Medicals, Andong National University

<sup>2</sup>Agriculture Science and Technology Research Institute, Andong National University, Korea

Bees are essential for ecosystem due to their crucial role in providing pollination services. Although there has been significant research on the commercial use of the genera *Bombus*, *Apis*, and *Osmia*, the valuation of wild bees, such as *Andrena* and *Lasioglossum* are insufficient. Pollinators and plant interact with each trait and rewards. This study compared the flower visiting insect diversity to congener trees, *Prunus davidiana* (Pd) and *P. salicina* (Ps) and their flower traits. We selected both trees which bloom at the same time in the close locations. Also, we analyzed the flower abundance and collora types, amount of pollen, and nectar, and the amino acid contents. Results revealed that *Apis cerana*, *Apis mellifera*, *Lasioglossum* spp. and *Andrena* spp. were found pollinating Pd, but only *Lasioglossum* spp. and *Andrena* spp. for Ps. The amount of flowers, pollen and nectar in Pd and Ps was measured at 7,620±970.21, 9,437±884.72, 1107±19.12, and 847±18.15, 0.33±0.06, 0.39±0.04 respectively. In terms of flower abundance, the amount of nectar was not statistically significant, but pollen of Pd were higher than Ps. In amino acid analysis, Valine, leucine, Phenylalanine and Histidine were higher in Pd, but Lysine and Arginine were higher in Ps. This shows a tendency to prefer higher pollen quality in congener trees that bloom simultaneously in close locations.



### OP-217

#### INSIGNIA-EU pilot study: botanical identification of pollen through ITS2 metabarcoding

M Alice Pinto<sup>1</sup>, Andreia Quaresma<sup>1</sup>, Robert Brodschneider<sup>2</sup>, Kristina Gratzner<sup>2</sup>, Fani Hatjina<sup>3</sup>, Ole Kilpinen<sup>4</sup>, José Rufino<sup>5</sup>, Flemming Vejsnæs<sup>4</sup>, Jozef Van Der Steen<sup>6</sup>, Alexander Keller<sup>7</sup>

<sup>1</sup>CIMO, SUSTEC, Instituto Politécnico de Bragança, Portugal

<sup>2</sup>Institute of Biology, University of Graz, Austria

<sup>3</sup>Ellinikos Georgikos Organismos DIMITRA, Greece

<sup>4</sup>Danish Beekeepers Association, Denmark

<sup>5</sup>CeDRI, SUSTEC, Instituto Politécnico de Bragança, Portugal

<sup>6</sup>Alveus AB Consultancy, Netherlands

<sup>7</sup>Faculty of Biology, Ludwig-Maximilians-Universität Munich, Germany

While classical palynology has been the method of choice to assess the botanical diversity of bee-collected pollen, DNA metabarcoding is emerging as a powerful alternative that is able to achieve high taxonomic identification accuracy. Moreover, DNA metabarcoding allows the analysis of hundreds of samples in a single high-throughput sequencing run, therefore offering unprecedented scale for citizen science projects. The pilot study of the European citizen science project INSIGNIA-EU (<https://www.insignia-bee.eu/>) included the analysis of 60 pollen samples collected biweekly from pollen traps between June and July of 2022 in Austria, Denmark, and Greece. A total of 41 families and 96 genera were detected in the three countries. Austria and Denmark showed similar floral distributions, with Fabaceae (23% and 30%, respectively) and Trifolium (19% and 18%, respectively) being the most abundant family and genus. In Greece, the most abundant family was Rosaceae (18%) and the most abundant genus was Rubus (19%). In each country, the top-5 families accounted for over 65% and the top-5 genera accounted for over 50% of the total relative abundance. The Shannon index showed that Austria (median=1.5) had lower diversity at the family level when compared to Denmark (1.7) and Greece (1.7); Yet, at the genus level, the three countries exhibited similar values, ranging from 1.6 for Austria to 1.8 for Greece. The temporal analysis of the data showed that the top-5 taxa were detected at each sampling round, although at varying relative abundances. The only exception was Brassica, in Denmark, which had a >30% relative abundance in the first sampling but then it became rare in the later samplings. This study shows that even though honey bees visit a high number of plant taxa, only five explain >50% of their pollen diet. Moreover, although Greece is known as a hot spot of diversity, three of the top five families are shared with the two northern countries.

### OP-218

#### Botanical sources associated with honey production by *Melipona mandacaia*

Tania Maria Sarmento Silva<sup>1</sup>, Yan Lima<sup>2</sup>, CATIANA Melquiades<sup>3</sup>, Francisco Israel Lopes Sousa<sup>3</sup>, GERLANIA Sarmento Verissimo<sup>4</sup>, Eva Sarmento Da Silva<sup>3</sup>

<sup>1</sup>São Paulo State University, Botucatu, Brazil.

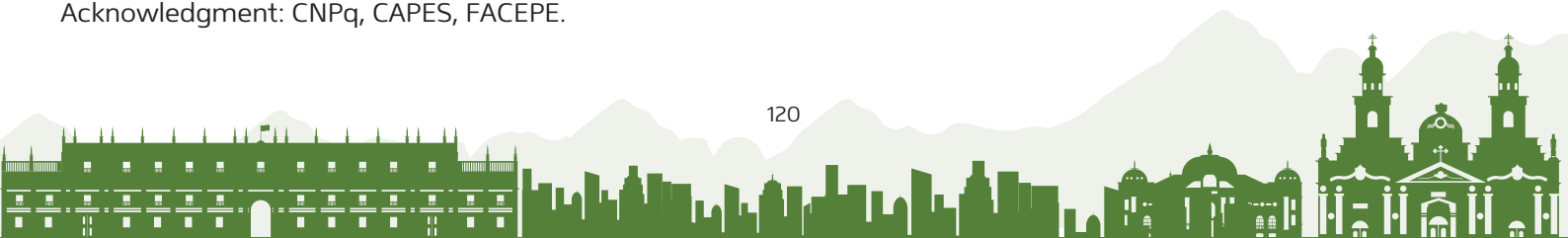
<sup>2</sup>Collegiate of Zootecnics, Federal University of São Francisco Valley, Petrolina, Brazil.

<sup>3</sup>Department of Chemistry, Federal Rural University of Pernambuco, Recife, Brazil

<sup>4</sup>University Hospital Lauro Wanderley, João Pessoa, Brazil.

The species *Melipona mandacaia* is one of the main species of stingless bees used for production of honey in the Brazilian semiarid region, being an endemic species. Thus, it is important to know the floral resources used by these bees for honey production in their native region of occurrence. Given the ecological, social and, economic importance of *M. mandacaia* in areas of the Caatinga biome, the objective of this study was to identify the pollen types present in samples of honey provisioned by *M. mandacaia*, as well as to characterize the botanical sources associated with the pollen types found. Honey samples were collected from hives located in a Caatinga area (Petrolina, Pernambuco State, Brazil). The samples were collected fortnightly for five months, during the period of higher honey production by these bees, called rainy period in the Brazilian semiarid region. The honey collected was diluted and acetolyzed, following standard protocols for melissopalynological analyses. The pollen types were identified based on the local palynoteca and specialized literature. Subsequently, the frequency of occurrence and distribution of pollen types during sampling was calculated. Thirty-five pollen types were identified in the honey stored by *M. mandacaia*, being grouped mainly in the botanical families Fabaceae (n = 6 pollen types), Malvaceae and Euphobiaceae (n = 4 pollen types, in each family). Most plants associated with the recorded pollen types are species that provide nectar as the main floral resource; in relation to the geographical distribution of the plants, they are mostly native Brazilian species and endemic to the semiarid region, such as: *Mimosa* spp. (Fabaceae), *Waltheria rotundifolia* (Malvaceae), and *Ziziphus joazeiro* (Euphobiaceae). Based on the pollen spectrum identified in the honey stored by *M. mandacaia*, we can see the importance of native flora in providing resources for honey production by these bees in their region of natural occurrence.

Acknowledgment: CNPq, CAPES, FACEPE.



### OP-219

#### Assessing the importance of natural areas on wild pollinators and honey bees in fruit crops in the central Mediterranean zone of Chile

Juan L Celis Díez<sup>1</sup>, Camila B García<sup>1</sup>, Manuel López Aliste<sup>2</sup>, Loreto Olgún<sup>1</sup>, Hans Moller Zapata<sup>1</sup>, Natalia Leiva Martínez<sup>1</sup>, Tamara Lillo<sup>1</sup>, Pablo Díaz Siefer<sup>3</sup>, Francisco E Fontúrbel<sup>2</sup>, Fernanda Montero Silva<sup>1</sup>

<sup>1</sup>Escuela de Agronomía, Pontificia Universidad Católica de Valparaíso, Quillota, Chile

<sup>2</sup>Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

<sup>3</sup>Centro Regional de Investigación e Innovación para la Sostenibilidad de la Agricultura y los Territorios Rurales, CERES, Quillota, Chile

Wild pollinators play a key role in ecosystem functioning and food security, supporting honey bees in pollinating crops. Despite their importance, wild insect populations are declining, mainly for the habitat loss due to agricultural expansion. The loss of natural habitats due to agricultural expansion is evident in the Mediterranean area of central Chile, a biodiversity hotspot for plants and wild bees and an important agricultural area. However, our current knowledge about the diversity of wild insects that could provide pollination services in this agricultural landscape is scarce. Natural habitat plays a key role in providing pollination services by wild insects for many crops worldwide. Therefore, understanding the role of remnant natural areas in the agricultural landscape of central Chile on the diversity and abundance of wild pollinators and honey bees' abundance is key to managing adequate crop pollination and productivity. The study was conducted in apple and sweet cherry orchards across Mediterranean central Chile, from the O'Higgins to the Maule region (34-35° S). The study design consisted of orchards within three different landscapes based on the proportion of natural areas within a 1km spatial buffer: (1) Agricultural Areas (AA) surrounded by less than 35% of Natural Areas (NA), (2) AA surrounded by 35 to 70% of NA, and (3) AA with more than 70% of NA around. The same landscapes were selected for both apple and sweet cherry orchards. Each orchard contained groups of 15x15 m plots where colored pan traps were located. In both crops, the results showed a significantly higher richness and abundance of wild pollinators with a higher proportion of natural area in the surrounding area. On the other hand, the surrounding areas had no significant effect on honey bees. In addition, the relative bee/wild insect ratio was higher in orchards with a lower proportion of natural area in the environment, and inversely, i.e., proportionally more wild insects, the higher the proportion of the natural area. Thus, our results suggest that conserving natural areas in the agricultural landscape is essential to maintain the diversity and abundance of wild pollinators in orchards to ensure crop productivity.

### OP-220

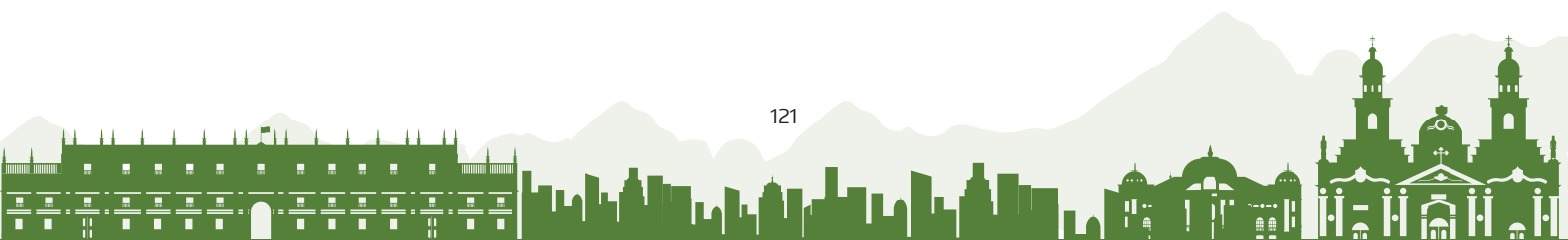
#### Biodiversity conservation and fruit production by native bees: estimation of floral constancy as an approximation to efficient and sustainable pollination

Sharon Rodríguez<sup>1</sup>, Isabel Acuna<sup>1</sup>, Franko Restovic<sup>1</sup>, Rodrigo Pizarro<sup>2</sup>, Catalina Escanilla<sup>1</sup>

<sup>1</sup>Center of Systems Biotechnology, Universidad Andrés Bello (CSB-UNAB), Santiago, Chile (1. - 3. - 5.)

<sup>2</sup>Science Department, San Benito High School, Vitacura, Santiago, Chile (4.)

The presence of native bees in orchards contributing to pollination and fruit production is one of the main reasons for conserving a diverse agricultural landscape and restoring native flora associated with crops. Both strategies promote greater diversity of native bees, carrying out complementary pollination tasks alongside honeybees, which could constitute a sustainable model for food production in the medium and long term. 464 species of native bees, belonging to 5 families (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae), have been described in Chile, many of which have been recorded visiting flowers of different crops. However, it is necessary to establish their participation in fruit tree pollination and verify pollen transport. To do this, we used the concept of floral constancy as an approach to the contribution of native bees to fruit production in almond (2), cherry (2), and avocado (2) orchards in the Metropolitan Region, where a hedgerow of native flora was established. During the 2020 and 2021 seasons, the abundance and richness of native bee families and their dispersion from the hedgerow were estimated at 30 points per orchard. Pollen was collected from 25 individuals per orchard to estimate pollen loads per crop. The implementation of the hedgerow in 2020 and its flowering in 2021 would explain the higher abundance and richness of native bee families in the orchards and an increase in floral constancy (mainly Halictidae, particularly on cherry). Bees showed dispersion in the orchard up to 350 m from the hedgerow. Significant differences ( $p < 0.05$ ) in abundance and floral constancy were recorded between both seasons. These findings raise the need to evaluate and value the participation of native bees as complementary pollinators to honeybees, together with the restoration of native habitats in simplified agricultural landscapes. Native flora and bees emerge as a link between conservation and production, so it is necessary to develop and implement landscape and orchard management strategies that promote sustainable fruit production.





### OP-221

## Climate change will provoke range drifting and may threaten some Amazonian bee species in the next 40 years, according to IPCC6

Vera Lucia Imperatriz-Fonseca<sup>1</sup>, Andre Luis Acosta<sup>2</sup>, Charles Fernando dos Santos<sup>3</sup>, Ricardo Caliari Oliveira<sup>4</sup>, Tereza Cristina Giannini<sup>1</sup>

<sup>1</sup>Biosciences Institute, Sao Paulo University, São Paulo, Brazil

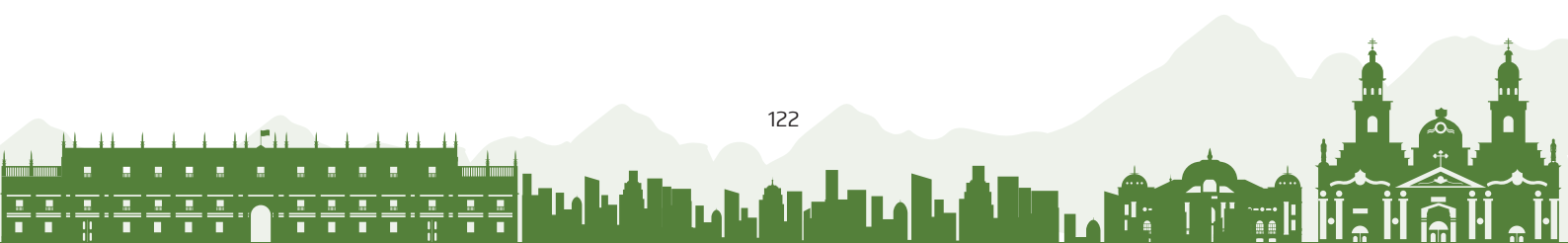
<sup>2</sup>Instituto Tecnológico Vale, Pará, Brazil

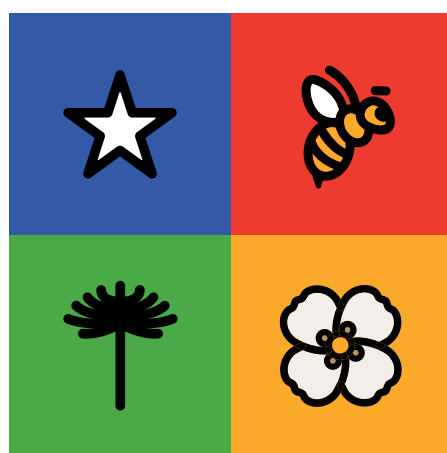
<sup>3</sup>Pontificia Universidade Catolica, RS, Brazil

<sup>4</sup>Autonomous University of Barcelona, Barcelona, Spain

<sup>5</sup>Universidade Federal do Pará, Pará, Brazil

Recent studies with bees in Oriental Amazon (Giannini et al., 2020) indicated that most bee species from 216 sampled in the preserved area studied will not find a suitable environment for survival in this area in 2050. Data were modelled with IPCC5 scenarios. Our question is how applying the climate change scenarios, using IPCC6 for species individually, could clarify the future climate impact on bee species. We choose among the bees studied previously some species used as pollinators or for meliponiculture: two bumblebee species (*Bombus transversalis* and *B. brevivillus*), two carpenter bees (*Xylocopa frontalis* and *X. grisescens*, seven stingless bees (*Melipona amazonica*, *M. interrupta*, *M. fasciculata*, *M. flavolineata*, *M. seminigra abunensis*, *M. seminigra pernigra*, *M. melanoventer*). Data sets were obtained using open-access databanks with occurrences reviewed by the authors. The scenarios showed range drifting, with some areas lost and the new regions suitable. Area suitability lost from current to 2040 ranged from 7% to 69,6% according to the bee species; present to 2060 ranged from 10,5% to 91,1%. It was possible to identify new species threatened by climate change, and *M. melanoventer* (will lose 91%) is the most endangered bee, followed by *M. seminigra pernigra* and *M. flavolineata*, that by 2060 will lose an area of 60,8% and 57,5%, respectively. Conversely, some species will find newly suitable areas, for instance, *Bombus transversalis*, *M. amazonica* and *M. interrupta*. The most critical period for the bees will be 2041-2060. The bees are essential pollinators, and most forest trees depend upon pollinators for reproduction. This scenario indicates the urgency of including pollinator monitoring studies in the bioeconomy of the Amazon Forest, which is essential for the Earth's climate equilibrium. There are many knowledge gaps in bee biology in the world's most biodiverse forest (only in Brazilian Amazon 183 stingless bee species are found). Improving knowledge of bee biodiversity must be a priority for subsequent years, when assisted pollination will be needed. Monitoring bees and their botanical resources with the climate scenarios is fundamental for attaining SDGs 2, 3, 13,15 e 17.





**APIMONDIA**  
**CHILE 2023**

# POSTER PRESENTATIONS



## Apitherapy

### PP-001

#### Pollen frequency of propolis samples from the insular zone of San Andres, Colombia

Guillermo Salamanca Grosso, Monica Patricia Osorio Tangarife

Mellitopalynological Research Group, Department of Chemistry, Faculty of Sciences. University of Tolima

Propolis is a natural product with a rubbery and sticky consistency. It is made by bees from resins, gums and plant exudations that have been modified after the addition of enzymes and secretions. It presents shades of brown, brown, brownish, reddish and green, and, in some cases, black, depending on the botanical and geographical origin. The diversity of plant species present in ecosystems is fundamental for the conservation of pollinating species particular, of bees, since they depend directly on the supply of pollen and nectar. The pollen spectrum of propolis is an indicator of the existing vegetation in a given biogeographic area and evidences in some way the plant-insect relationship in the search for subsistence resources for bee colonies. We evaluated five samples of red propolis collected in San Andres Island (Colombia) to determine the frequency and types of pollen present. The samples were subjected to separation and concentration, as well as sediment analysis by acetolysis. The work allowed the recognition of 46 pollen types from 29 botanical families, predominantly *Cyperus haematodes*, *Avicennia germinans*, *Conocarpus erectus*, *Dalbergia brownei*, *Laguncularia racemosa*, *Rhizophora mangle*, *Sesuvium portulacastrum*, *Cocos nucifera* and *Spondias mombin* and other less important types, such as *Morinda citrifolia*, revealed through their pollen frequencies. The families with the highest number of pollen types present corresponded to *Acanthaceae*, *Aizoaceae*, *Arecaceae*, *Combretaceae* and *Rhizophoraceae*. These results represent one of the first pollen analyses on Colombian propolis.

### PP-002

#### Propolis in dentistry. Uses and applications. Clinic cases

Rafael Felitti<sup>1</sup>, Nicolás Cardozo<sup>2</sup>

<sup>1</sup>Dr Rafael Felitti. Dentist and apitherapy. Dentist department of Apiter. Montevideo.Uruguay

<sup>2</sup>Laboratorio Apiter SA

Propolis is a resinous product that is collected from plants by bees to cover holes and crevices in their hives. Propolis has potent antibacterial, antiviral, anti-inflammatory, wound healing, and anticancer properties. Propolis has been used therapeutically by humans for centuries, including the treatment of dental caries and mouth infections. Propolis is an antibacterial, anti-inflammatory, and analgesic product and we can use it for dental treatments like root canal treatment and antibacterial, operative dentistry because it can stimulate the stem cells of the pulp and in periodontal treatments for irrigating the periodontal pockets because propolis can kill anaerobic and aerobic bacteria and we can use it for candidiasis treatments because propolis is an antimicrobial.

The importance of using standardized propolis for these treatments is very important because we need the same quality all the year and can be an opportunity to develop new biomaterials for dentistry uses. Propolis is an excellent and natural alternative for use in dentistry.

### PP-003

#### Protective Effect of Propolis Mixture mixed with Herbs against Helicobacter Pylori-Induced Stomach Ulcer in C57BL/6 Mouse

Jiheon Lee<sup>1</sup>, Hadong Kim<sup>1</sup>, Seoungwan Lee<sup>1</sup>, Na Rae Kim<sup>2</sup>, Mee Ree Kim<sup>2</sup>

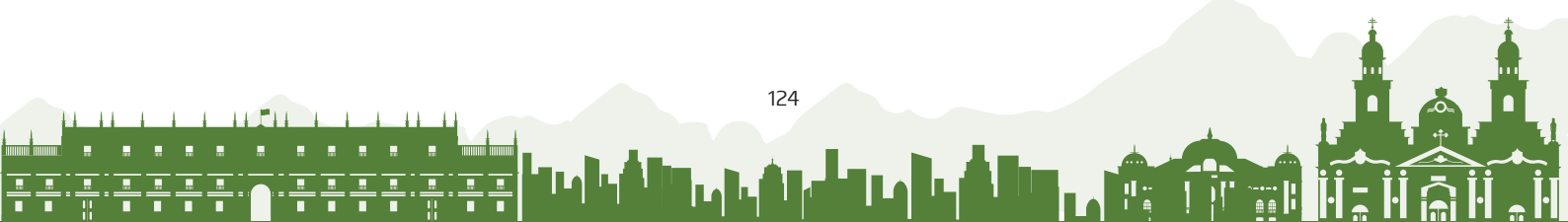
<sup>1</sup>Propolis R&D center, Seoul Propolis, Seoul, South Korea

<sup>2</sup>Department of Food & Nutrition, Chungnam National University, Daejeon, South Korea

Digestive ulcers are a common digestive disease that affects at least 4-5% of the world's population at some point in their lifetime. Propolis, a natural substance known for its antioxidant and antimicrobial properties against oral bacteria, has been used as a health functional food. However, its effect on suppressing stomach damage is not known yet.

In this study, after infecting C57BL/6 mice with *Helicobacter pylori*, a major pathogen for peptic ulcer, the antimicrobial activity of Propolis Mixture mixed with natural materials, such as olive leaf extract and licorice extract, was measured through the pathological histologic findings. *H. pylori* colonization, expression analysis of pre-inflammatory cytokine-related genes, and oxidative stress analysis.

As a result, the propolis mixture suppressed the ulceration on the damaged gastric mucosa in C57BL/6. Especially, compared to propolis only, the propolis mixture mixed with olive leaves extract and licorice extract showed significant anti-*pylori* adhesion, antioxidant and anti-inflammatory effects. Therefore, based on these results, we suggest that the proposed Propolis Mixture has potential to be developed as a material for protecting against stomach ulcers.



## Apitherapy

### PP-004

#### Use of bee products in the treatment of acute and chronic wounds. Clinical cases

##### Stefan Stangaciu

Romanian Society of Apitherapy, Phytotherapy and Aromatherapy

Beehive products, such as honey, propolis, royal jelly, and bee venom, have been used for centuries in traditional medicine for the treatment of various diseases, including wounds. Research done in the last 50 years has confirmed their efficacy in wound healing, particularly in the treatment of burns and chronic infected wounds.

Honey has antimicrobial and anti-inflammatory properties and has been shown to promote wound healing by reducing inflammation and stimulating tissue regeneration. Propolis has been found to have potent antimicrobial, anti-inflammatory, and antioxidant effects, making it a promising treatment for chronic infected wounds.

We observed, in our own clinical experience, that the beehive products are effective in the treatment of burns and chronic infected wounds.

We found that honey has significantly reduced the healing time and possible complications of burn wounds compared to conventional treatments. Propolis, combined with honey and thyme oil, has been found to be effective in the treatment of diabetic and decubitus ulcers. Royal jelly has also been shown to improve the healing of diabetic foot ulcers. Bee venom therapy has been used successfully in the prevention and treatment of chronic wounds, especially those specific to arteritis obliterans.

In conclusion, beehive products are promising natural treatments for acute and chronic infected wounds. They possess potent antimicrobial, anti-inflammatory, and antioxidant properties and have been shown to stimulate tissue regeneration and enhance wound healing.

Further research is needed to explore the full potential of these products and to determine their optimal use in wound care.

### PP-005

#### Green Brazilian Propolis Oil Extract

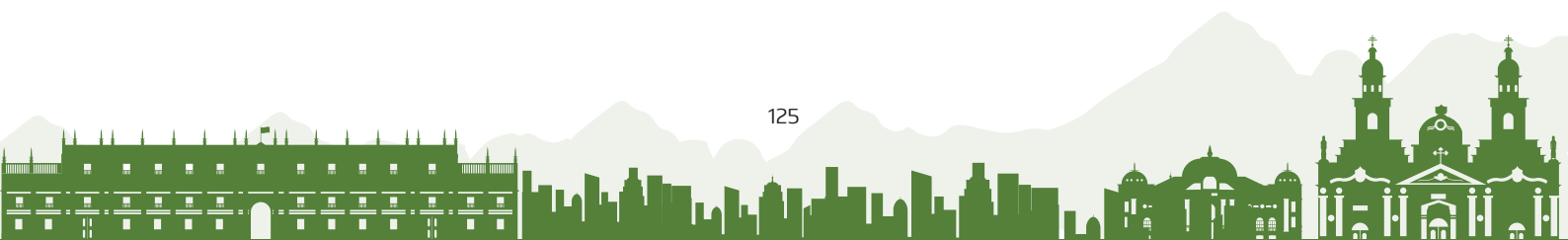
##### Rafael Felitti<sup>1</sup>, Nicolás Cardozo<sup>2</sup>

<sup>1</sup>Dr Rafael Felitti. Dentist and apitherapy. Dentist department of Apiter. Montevideo.Uruguay

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### PP-006

#### Scientific-based evidence supporting the remarkable properties of honey-bee venom

Marie Agnes Courty<sup>1</sup>, Claude Grison<sup>2</sup>, Jean Michel Martinez<sup>1</sup>, Erwan Oliviero<sup>3</sup>, Franck Pelissier<sup>2</sup>, Rachel Pflieger<sup>4</sup>, Mariana Timm<sup>3</sup>, Catherine Flurin<sup>5</sup>, Boris Cabrol<sup>6</sup>

<sup>1</sup>PROMES UPR 8521 CNRS, Univ. Perpignan, Tecnosud, Perpignan, FR. marie-agnes.courty@promes.cnrs, France

<sup>2</sup>Chimeco, UMR 5021 CNRS, 1682 Rue de la Valsière, 34790 Grabels, France

<sup>3</sup>PROMES UPR 8521 CNRS, Univ. Perpignan, Tecnosud, Perpignan, FR. marie-agnes.courty@promes.cnrs.fr

<sup>4</sup>ICGM UMR 5253, Univ. Montpellier, 34095 Montpellier, France

<sup>5</sup>ICSM UMR 5257 – CEA / CNRS / UM / ENSCM Site de Marcoule, Bagnols sur Cèze, France

<sup>6</sup>Société Abeilles Santé, 100 Rue des Moulins, 65700 Maubourguet, France

<sup>7</sup>Association Joseph-Sauvy, Chem. du Mas Taillant, 66430 Bompas, France

We report here the in situ multi-analytical protocol for studying polymer solid components that form from the venom liquid glands when abruptly ejected by honey-bees. The complete assemblage of the propelled components was meticulously collected using a hollowed-out box with conducting copper wires. Silicon, glass or polymer supports were alternatively used for adjustment to the analytical characterization. Three remarkable results were obtained by successive collects in 2021 and 2022 from different hives in southern France and Ibiza (Canary Islands).

(1) We can now describe the venom ejection by honey-bees to comprise two distinctive parts: (a) a head consisting of dense, spheroidal aggregates, often sprinkled with multi-sized microspherules, embedded in a heterogeneous, polymeric matrix, defined here as the propelled bullet; (b) an elongated, thin finely cracked, homogeneous, translucent polymeric matrix with residual vesicles and nanostructured groove-marks at its surface, considered by this morphology as the post-ejection tail.

(2) The respective composition of the bullets and of the related tails appeared to greatly vary: (a) between bees of the same hive in the same conditions; (b) between hives of the same apiary; (c) between successive collects of the same apiary depending upon weather conditions and meteorological events. The meticulous study of this compositional variability emphasizes the capacity of certain honey-bees to produce high-energy venom ejection. This is defined both by the particular composition of the ejected bullet - i.e. in terms of magnetite spherule content and abundance of scattered metal nanoparticles in the polymeric matrix - and the morphology of the bullet-tail couple.

(3) A correlation was observed between a long-lasting episode of highly ionized atmospheric aerosols in 2022 and the venom injection by the honey-bees within the polystyrene frame.

The nanostructuring of the bullet-tail couple and its nanoparticle content suggest that the honey-bees collected exceptional resources that were only available during this meteorological situation. Their final characterization as part of the research project LINA will soon elucidate further the physical mechanisms explaining this high-energy storage at nanolevel and their use by honey-bees. These original results are viewed to open challenging perspective to optimize the performance of apitherapy.

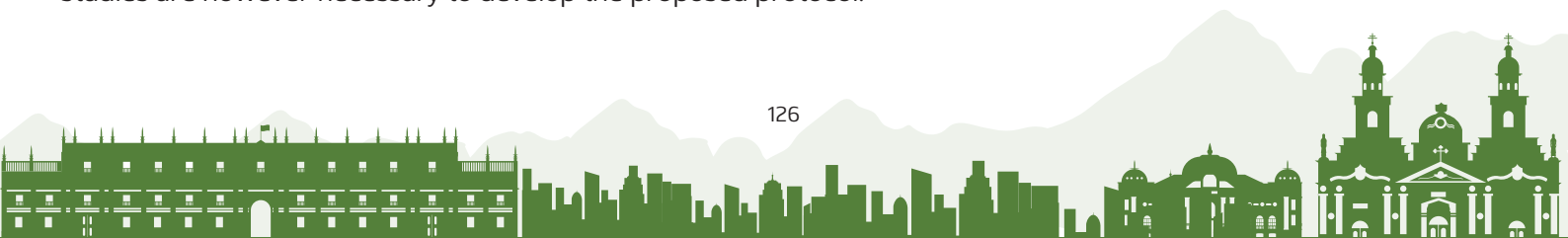
### PP-007

#### Proposal of a protocol for the use of honey dressings in pet medicine

Philippe Garcia

Association Francophone D'Apithérapie

Wounds are a very frequent reason for consultation in pet veterinary medicine. Conventional treatment protocols use expensive dressings and products, which are not always financially accessible to pet owners. The aim of this paper is to propose a protocol for the use of honey dressings in pets. After a reminder of the natural evolution of a wound, the protocols commonly accepted in classical medicine recommend surgical or mechanical debridement and then management in a more or less humid environment depending on the stage of healing. Honey, due to its physicochemical and bacteriostatic properties, has a definite interest in the four phases of wound healing: necrosis, detersion, granulation and epidermisation. Based on numerous clinical cases that we encounter in our daily practice, we have been able to establish a protocol for making dressings. The necessary material includes a rinsing solution such as ringer lactate, honey of apitherapeutic quality and fatty tulle for the contact layer, cellulose wadding and/or an American compress for the absorbent layer, a crepe strip and an adhesive strip for the protective layer. After surgical or mechanical debridement with the rinsing solution, the contact layer with honey is applied, followed by the absorbent layer and finally the protective layer. The dressing is renewed once or twice a day in the necrosis or detersion phase, then every two or three days in the granulation phase and finally once every four to five days in the epidermisation phase. This protocol, which is the result of our experience, gives us complete satisfaction and lays the foundations for practical management of wounds with honey in pet veterinary medicine. It probably needs to be improved. To date, we have only encountered one pseudo-failure in a cat due to osteomyelitis, probably associated with underlying nerve damage. The centralisation of the field experiences of veterinary apitherapists, a standardisation of the quality criteria of honeys that can be used in healing and more important scientific and clinical studies are however necessary to develop the proposed protocol.





## Apitherapy

### PP-008

#### Proposal of apitherapeutic treatment protocols for some major syndromes in dogs in the context of the problem of antibiotic resistance

Philippe Garcia

Association Francophone D'Apithérapie

Antibiotic resistance is the major health problem to be solved according to the WHO recommendations. The reduction of antibiotic prescriptions in both humans and animals has become a priority. There is an urgent need to develop scientifically validated alternative treatments. Many scientific publications show the interest of propolis in this indication. Unfortunately, most of the studies do not propose protocols that can be used directly in veterinary clinical practice, and the laboratory results are sometimes inconsistent. In small animal veterinary practice, many pathologies are very consuming of antibiotics, such as otitis and digestive disorders. The aim of this study is to propose, based on literature data, alternative treatment protocols to antibiotics using standardised propolis extracts possibly combined with honey. These treatment protocols were used on clinical cases recruited in a veterinary clinic. In the clinical cases recruited, we obtained in most cases an improvement and in a certain number of cases a cure without having to resort to antibiotics. Apitherapy therefore seems to be an interesting solution in the fight against antibiotic resistance.

### PP-009

#### Effect of honey and adulterated honey consumption on the health of wistar rats

Jose-Carlos Tapia Rivera<sup>1</sup>, Alvaro De la Mora<sup>2</sup>, Francisca Contreras Escareño<sup>1</sup>, José María Tapia González<sup>1</sup>, Jose Octavio Macias-Macias<sup>1</sup>, Andrea Ayala Gutiérrez<sup>1</sup>, Ernesto Guzmán Novoa.<sup>2</sup>

<sup>1</sup>Department of Ciencias de la Naturaleza, Universidad de Guadalajara, Jalisco, Mexico

<sup>2</sup>Department of bee research, University of Guelph, Ontario, Canada.

Honey is one of the most adulterated food products around the world, the main ingredients used to adulterate honey have been described as harmful to humans, for which this study analyzed the effects of consuming honey and adulterated honey on the health of Wistar rats. This study investigated the effects of consuming natural and adulterated honey on the health of Wistar rats. 21 male rats aged 8-10 weeks were divided into three groups: one group received a daily solution of honey, another group received a solution of 70% rice syrup and 30% honey, and the third group was not given any sweetener. Food and water were given to all groups freely. It was observed that the control group consumed the most food  $p < 0.05$ , and had the highest glucose levels at weeks 2 and 8  $p < 0.05$ . However, there were no significant differences in the other variables, such as body weight, height, BMI, and antioxidant capacity in the plasma. This study concluded that the consumption of honey and adulterated honey did not affect the BMI or glucose levels of the rats.

### PP-010

#### Study of the influence of the location of the bee venom extraction trap on its composition

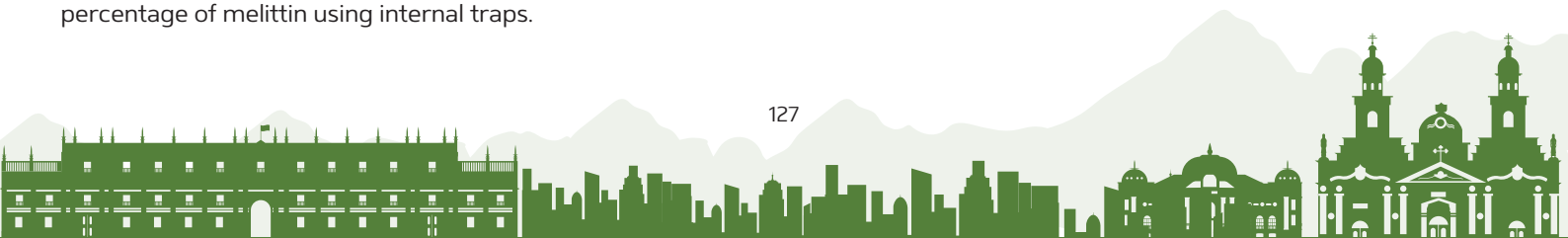
Agustín Pérez Graña<sup>1</sup>, Valentina Rodríguez Batista<sup>2</sup>, José Anzola<sup>2</sup>, Zonia Martínez<sup>3</sup>, Pablo Juri<sup>2</sup>, Enrique Nogueira<sup>2</sup>, Catherine Fagúndez<sup>2</sup>

<sup>1</sup>Facultad de Agronomía, Universidad de la Republica, Montevideo, Uruguay

<sup>2</sup>Facultad de Veterinaria, Universidad de la Republica, Montevideo, Uruguay

<sup>3</sup>Instituto de Farmacia y Alimentos, Universidad de la Habana, Habana, Cuba

Apitoxin is a bee product made up of dozens of bioactive substances that begin to be synthesized from the time the adult bee emerges until it completes its venom sac about three weeks later. Commercial apitoxin is obtained using an electrical current emitting equipment connected to the traps that have a metallic grid with a glass attached, so that when the bee's lands on the circuit it closes it and receives a small electric shock that triggers a sting, depositing the venom without killing the bee. These traps can be located inside or outside the hive, the bees that will access one or the other will be younger or more mature due to temporary polyethism. The goal of this work was to evaluate if the place where the trap is placed affects the composition of the apitoxin. The experiment was performed in the Experimental Field of the Faculty of Veterinary using 20 hives, all with an estimated population of over 40,000 adult bees. They were divided into Group IT (internal traps) and Group ET (external traps), the extraction was performed in the afternoon for 30 minutes. The glasses corresponding to each colony were identified and the HPLC percentage of melittin and apamin of the harvested apitoxin was quantified in each hive in the experiment. The results were evaluated using a variance analysis for a completely randomized design and significant differences in melittin were found, with IT 72.63%, ET 62.37% ( $p$ -value 0.036) and in apamine, IT 1.55%, ET 1.47% ( $p$ -value 0.005). These differences are possibly caused by a different speed of the synthesis of the different components of the apitoxin, so that the composition would vary according to the age of the bees and therefore the composition of the harvested apitoxin is going to vary according to whether the traps are visited by younger bees (IT) or mature bees (ET) which opens the possibility to direct the production of the apitoxin with a higher percentage of melittin using internal traps.



## Apitherapy

### PP-011 Utility of honey-based dressings for the management of chronic wounds

Andrii Dinets<sup>1</sup>, Maksym Gorobeiko<sup>2</sup>, Oleksandr Nykytiuk<sup>3</sup>, Olesia Lynovytska<sup>3</sup>, Victoria Zdorna<sup>1</sup>, Galina Davidova<sup>1</sup>, Svitlana Hotska<sup>1</sup>, Volodymyr Postoienko<sup>1</sup>

<sup>1</sup>Department of Apitherapy, Prokopovych National Beekeeping Research Institute, Kyiv, Ukraine

<sup>2</sup>Department of Surgery, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

<sup>3</sup>Department of Physical Therapy, Kyiv Agrarian University, Kyiv, Ukraine

#### Background

Healing of chronic wounds is a complex process and worldwide problem for patients with gunshot wounds, diabetes mellitus, chronic venous insufficiency. Wound trophic disorders, neuropathy, microbial contamination and other negative factors lead to the delay of the natural healing process and application of the standardized pharmacological agents is not always associated with a good results. One of the major issues with ulcers is formation of microbial biofilms that minimize the effectiveness of standard topical treatment by various wound-healing agents with a directed effect on one or another pathogenetic factor of the wound process. In such circumstances, apitherapy is considered as a perspective approach for the management of chronic wounds. Honey-based dressing could be used as an additional element for the treatment of chronic wounds.

The aim of the study was to develop and test the utility of using a honey-based dressings for the management of chronic wounds in diabetic foot.

#### Patients and methods

A 15 patients diagnosed with diabetes mellitus type 2 (DMT2) and diabetic foot were involved in the study. DMT2 was managed according to American Diabetes Association standards. These patients did not show good results with wound healing by using standard treatment options. For these individuals, we added dressings impregnated with honey, pollen and bee chitosan in addition to the standard treatment regimen twice a day for 20 days.

#### Results

There were 10 women (67%), 5 men (23%). All patients had diabetes compensation (target fasting glucose levels up to 7 mmol/l, 2 hours postprandial up to 10 mmol/l). A significant improvement of the wound process was observed in 14 (93%), which was manifested by a decrease in the volume of excision of necrotic tissues during dressings, an improvement in blood supply, a decrease in exudation, and the intensive appearance of granulations. From the beginning of the course of treatment, complete healing of the wounds occurred within 33.4 days (mean).

#### Conclusions

The use of dressings impregnated with with honey, pollen, bee chitosan seems to be an effective approach in addition to the standard schemes of treatment of the diabetic foot, which is associated with good treatment results.

### PP-012 Topical Propolis in Diabetic Foot

Héctor Tito Leoni<sup>1</sup>, Nicolás Cardozo<sup>2</sup>

<sup>1</sup>Instituto de Rehabilitación Psicofísica de Bs. As., CABA, República Argentina

<sup>2</sup>Laboratorio Apiter SA

#### INTRODUCTION

Natural therapies for the treatment of wounds have a proven therapeutic efficacy. Their availability, accessibility and simplicity allow them to be combined with other therapies.

Diabetic foot is an infection, ulceration and destruction of deep tissues associated with neuropathy and different degrees of peripheral vascular pathology in lower limbs. Due to the difficulty in the management of these lesions and the need to find ways of effective treatments readily available, we directed that search towards products of natural origin, such as Propolis.

#### OBJECTIVES

to demonstrate that the use of Propolis is effective for the treatment of leg and foot ulcers in patients with diabetes.

#### METHODOLOGY

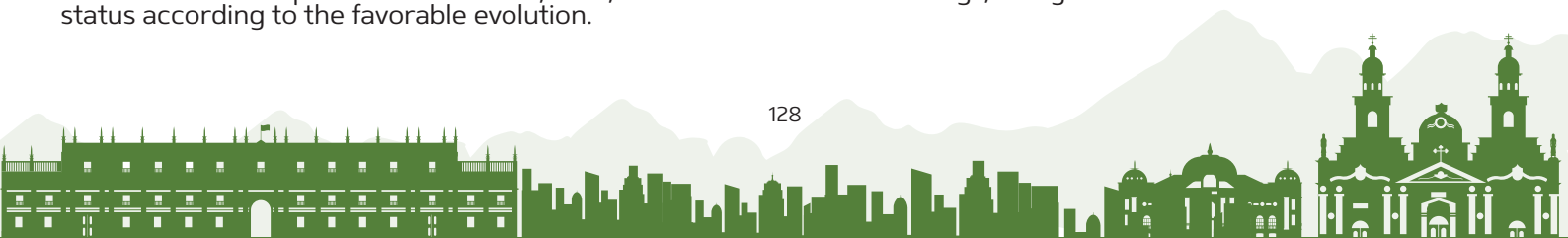
A descriptive study was conducted from March 2018 to March 2019, in 25 patients with an average age of 50 years. All presented complex wounds of diabetic origin, in which pharmaceutical forms with topical Propolis were used, for their treatment.

#### RESULTS

Of the 25 patients included, 20 resolved their lesions, 4 are still in the study, 1 patient left the study due to resistance to treatment. All were treated with topical Propolis exclusively or as an adjuvant to other procedures such as wet cure, PTN or skin grafting.

#### CONCLUSION

In complex pathologies such as diabetic foot and diabetic ulcerative lesions of the lower limb, the use of Propolis was beneficial, favoring the healing process. The socioeconomic impact lies in the easy availability and good cost/benefit ratio, high tolerance and adherence to treatment, decrease in the number of cures due to the prolonged interval between cures, shorter hospitalization time, reduction of expenses for the system. For the patient, being favored in the reduction of expenses in medicines, time, transfers and loss of earnings, being able to resume his social and labor status according to the favorable evolution.



## Apitherapy

### PP-013 Comparison of the shelf life of honey shampoo with propolis as a preservative versus honey shampoo without preservative

Adela Castañeta

Adela Castañeta Castillo

Eng. Adela Castañeta Castillo

Master in Business Administration, Agronomist, Diploma in Chemistry and Processes, Diploma in Corporate Social Responsibility with sustainability,

#### 1. Introduction

There are several brands of shampoo that offer these hair cleansing products containing parabens, which are used as preservatives to prevent the growth of bacteria and fungi, they have an estrogenic effect on the body. The purpose of this research is to evaluate propolis as an alternative to the use of parabens.

#### 2. Objectives

• Compare the shelf life of honey shampoo with propolis as a preservative versus honey shampoo without preservative.

The hypothesis is as follows:

• Alternative hypothesis: "Honey shampoo with propolis as a preservative has a significantly longer shelf life than shampoo without preservative."

#### 3. Methods

It is exploratory research. The method used was the random sampling of 120 shampoos to evaluate every 3 months the duration of the product, performing sensory analysis of them, taking into account the appearance, aroma, texture of honey shampoo (2%). The dose used as a preservative is 1% propolis (30%) in the shampoo. Likewise, a sample of 120 honey shampoos (2%) without preservative is made. During the sensory evaluation stages, physical, chemical and stability analysis is performed.

#### 4. Results

In the initial evaluation both products show good appearance.

At the third month the honey shampoo without preservative month presents bubbles and change of color in the shampoo.

The honey shampoo with propolis as a preservative in the third month, the sixth, the ninth presents stability, in the twelfth month there is a change in color and in its aroma.

#### 5. Conclusions

Honey shampoo with propolis (30%) as a preservative in a dose of 1%, can last for 9 months, in this time the shampoo retains its quality and effectiveness. The honey shampoo without propolis at the third month presents bubbles and color change so its duration does not reach 3 months.

### PP-014 Use of propolis solutions in SARS-CoV-2 patients and contacts

Amelia Cristina Tor

Amelia Cristina Tor, Sociedad Apícola Uruguaya, Montevideo, Uruguay.

From March 2020 to December 2022, hydroalcoholic solutions of propolis with concentrations of 20% and 30% were supplied. Corresponding to each participating country (Mexico, Chile, Peru, Ecuador, Colombia, Brazil, Paraguay, San Salvador, Uruguay, Spain) under treatment protocol, during 2020.

30 drops were administered 3 times a day to each patient.

Inhalation with nasal mask  
5 to 15 drops in saline solution  
3 to 4 times a day

Propolis sprays 3 to 4 times a day.

From 2021 to 2022 we supply 120 drops daily in 3 doses of 40 drops each.

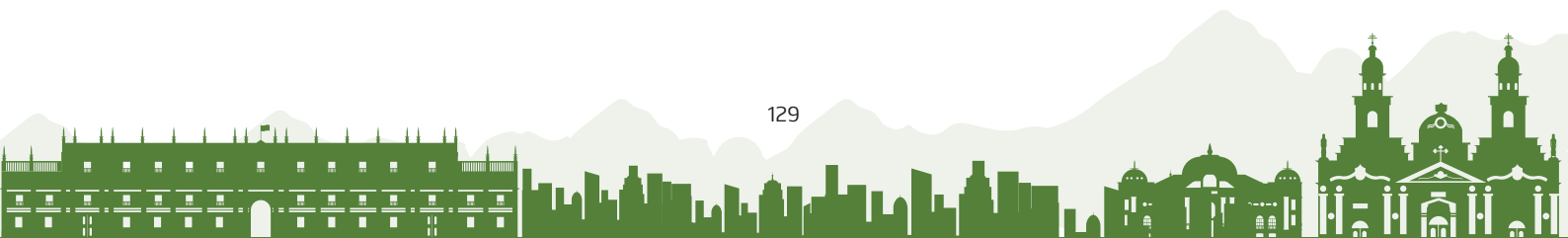
Intra-domiciliary contacts and people at risk  
25 drops of propolis solution 2 times a day.

The age group studied was from 2 to 92 years in the period from 2020 to 2022. These people had previous pathologies or not.

Pregnant women of 4 and 7 months of gestation were treated.

Treatment began 10 days after symptom onset in each patient.  
The patients presented complete improvement without subsequent complications.

There were no deaths.



### PP-015 Biological properties and nutritional values of bee products

Hesham El Seedi<sup>1</sup>

<sup>1</sup>Pharmacognosy Group, Department of Pharmaceutical Biosciences, Uppsala University, Biomedical Centre, Box 591, SE 751 24 Uppsala, Sweden

<sup>2</sup>International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang 212013, China

Bee products are often sold as nutritional supplements and/or health products, with potential anticancer, antimicrobial, antioxidant, anti-nociceptive, and anti-inflammatory activities. Bee products have played major roles in traditional medicine. Secondary metabolites from an immense diversity of bee products such as honey, pollen, propolis, royal jelly or bee venom represent a huge repository of chemical biodiversity, providing a renewable and rewarding source of therapeutic substances. Bee products especially polyphenols have neuroprotective actions via quenching the reactive oxygen species that cause neurotoxicity and aging as well as combating the pathological deposition of misfolded proteins. This presentation will highlight the impact of bee products and its ingredients against neurodegenerative diseases [1].

Furthermore, we will demonstrate the importance of bee pollen and bee bread as vital products of health care. Bee bread is used mainly for their anti-cancer, anti-microbial, anti-fungal effect [2,3]. Additionally, the antiviral properties of propolis will be discussed as potential therapeutic treatment for COVID-19 disease [4].

Bees as an insect play a crucial role in crop pollination alongside other animal pollinators. Bees contribute to the global food supply via pollinating a wide range of crops, including fruits, vegetables, oilseeds, legumes, etc. Bee pollination improves the quality and quantity of fruits, nuts, and oils. However, bee colonies are faced with many challenges that influence their growth, reproduction, and sustainability, particularly climate change [5,6]. The talk will tackle the contribution of bees to crop pollination and the limitations facing the pollination.

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### PP-016 Stingless bee´s honey: an ancestral food validated for its functional properties

Virginia Salomón<sup>1</sup>, Gerardo Pablo Gennari<sup>1</sup>, Luis Maldonado<sup>1</sup>, Cintia Romero<sup>2</sup>, Nancy Roxana Vera<sup>3</sup>

<sup>1</sup>Instituto Nacional de Tecnología Agropecuaria (INTA). EEA Famaillá, 4132, Famaillá, Tucumán, Argentina

<sup>2</sup>PROIMI-CONICET (Planta Piloto de Procesos Industriales Microbiológicos). Avenida Belgrano y Pasaje Caseros, 4000, Tucumán, Argentina.

<sup>3</sup>Facultad de Bioquímica, Química Y Farmacia. UNT. Ayacucho 471, 4000, Tucumán, Argentina

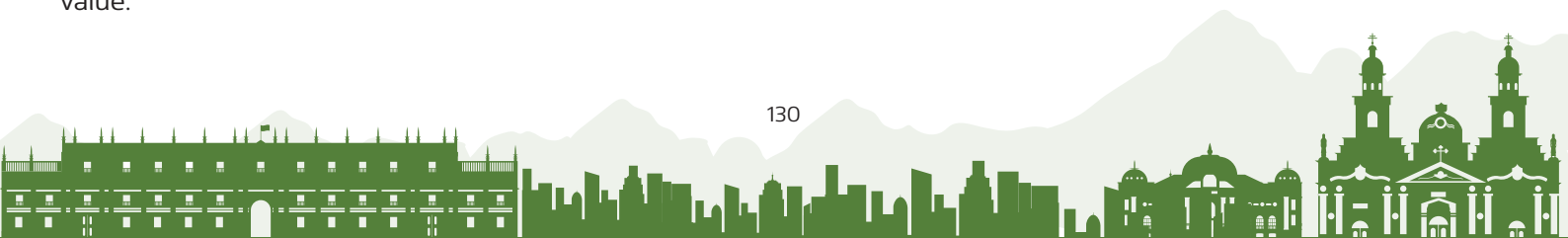
Despite all the advantages that the consumption of meliponid honey entails, insects of the Apidae family (Hymenoptera: Apidae: Meliponini), published studies on these species are very scarce. In this work we study *Tetragonisca fiebrigi* and *Scaptotrigona jujuyensis* honeys produced in Tucumán, Argentina. Physical-chemical and melisopolynological analyzes were carried out and we evaluated in vitro its antioxidant properties and in vivo its analgesic, anti-inflammatory, antitussive, expectorant activity and its safety. Honeys were classified as dark with variable total phenol values; the two species were differentiated by color, pH and UV spectroscopy determinations. The predominant pollen species were *Eucalytus* sp. and *Conium* type. The profile of carbohydrates by HPLC was similar for both honeys; the main ones being fructose and glucose. gallic, caffeic, coumaric, ferulic and cinnamic acids and flavonoids: quercetin and kaempferol was quantified.

In vivo studies with experimental animals, they exhibited central and peripheral antinociceptive action, acute and chronic anti-inflammatory activity. They prolonged the latency period and inhibited cough frequency and showed no signs of toxicity. Both exhibited antioxidant activity in vitro.

The microbiological quality of the honey and the pollen was determined were classified as fit for consumption.

The microbial ecology of honey, pollen, and bee gut was evaluated. The phylum Bacillota (*Bacillus* sp. and *Lactobacillus* sp.) and *Pseudomonadota* were dominant. The hydrolytic potential of 168 isolated strains was studied. Highlighting the proteolytic and amyolytic activities. Three isolates were selected and their growth in bile salts, antimicrobial activity and the production of an extracellular matrix (exopolysaccharide-EPS) were evaluated. *Bacillus* sp. 4A produced an exopolysaccharide, this was partially purified, and identified as levan. Studies of its properties were carried out: emulsifying, antimicrobial and its prebiotic activity against *Lactobacillus* sp.

Additionally the resistance of the selected strain to the simulated gastrointestinal tract was studied. Thus, a levan-type exopolysaccharide with the ability to emulsify hydrogels and prebiotic activity was produced from a potentially probiotic microorganism, isolated from the native microbial flora present in *S. jujuyensis* honey. Our results showed that native stingless bees honey is a source of bioactive compounds and microorganism with functional and nutritional value.



## Apitherapy

### PP-017

#### Phytochemicals from PhytoMed Honey with high docking affinity with SARS CoV-2 proteins and the traditional medicinal uses of their plant sources

Valma Roslyn Jessamy

Caribbean Institute for Sustainable Island Living

PhytoMed Honey® is a varietal of honey which was naturally produced by developing a proprietary planting scheme of tropical medicinal plants native to the Caribbean as the nectar and pollen source for honeybees *Apis Mellifera*. PhytoMed Honey was administered to PCR positive Covid-19 patients in a Human Covid Trial and patients symptoms improved after 72 hours with second PCR test negative.

Using HRMS 176 phytochemicals were identified in the honey and 35 are known to have anti-viral activity against other viruses. Molecular docking studies of the antiviral phytochemicals in PhytoMed Honey revealed the mechanism by which the honey was able to express activity against SARS-CoV-2. Twenty-three phytochemicals with the highest docking affinity ( $>-7\text{kal/mol}$ ) with main protease (7alh) were linked to their plant source.

Pharmacological studies have validated the use of these honeybee plants in traditional herbal medicine for treatment of a range of disease conditions. The combination of phytochemicals in PhytoMed Honey present a novel formulation for use of the medicinal plants. These plants are recommended as targets of conservation and further study on their seasonal variability in target phytochemicals, possibility to cultivate in nurseries and impact of climate change on their distribution in tropical wild forests.

### PP-018

#### Effect of the Ethanol Extract of Chilean Propolis on Senescent Rat Cartilage

Consuelo Arias Lattes<sup>1</sup>, Belgica Vasquez<sup>2</sup>, Luis A. Salazar<sup>3</sup>

<sup>1</sup>Escuela de Kinesiología, Facultad de Odontología y Ciencias de la Rehabilitación, Universidad San Sebastián, Santiago, Chile

<sup>2</sup>Facultad de Ciencias de la Salud. Universidad de Tarapacá Chile

<sup>3</sup>Centro de Biología Molecular y Farmacogenética, Departamento de Ciencias Básicas, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile

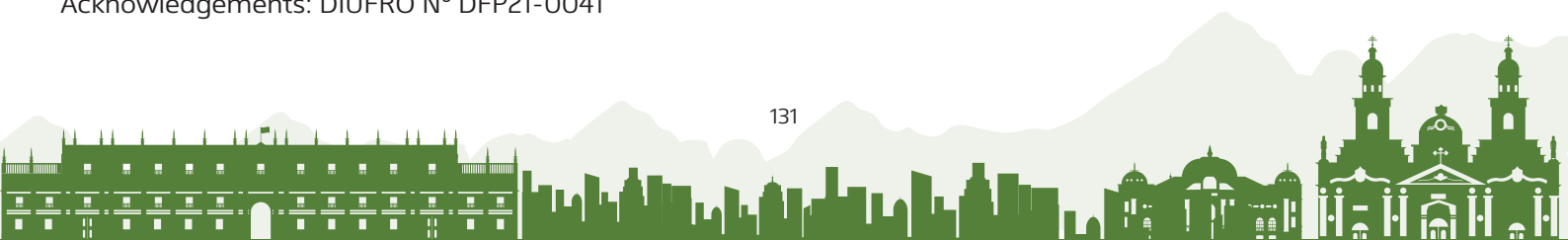
**INTRODUCTION:** As life expectancy increases, finding novel strategies that promote healthy aging becomes relevant. One of the most detrimental outcomes of aging corresponds to osteoarthritis (OA), a disabling, complex and multifactorial joint disease. To date, OA-modifying medications are still lacking, for this reason, searching for different therapeutic alternatives becomes necessary. Propolis is a resinous substance rich in polyphenols with multiple pharmacological properties that have been attributed to their antioxidant and anti-inflammatory capacity, which can reduce chronic inflammation associated with aging. For this reason, the main goal of this study was evaluated to evaluate if the *in vivo* treatment with ethanolic extract of propolis (EEP) could reverse the possible histological alterations generated in cartilage by aging, promoting the viability of chondrocytes and the maintenance of healthy cartilage.

**MATERIALS-METHODS:** 24 male Sprague Dawley (SD) rats of months old were divided into 3 groups. SR: senescent rats; SR-EEP: senescent rat treated with EEP and SR-V: senescent rat treated with vehicle. The treatment was carried out through oral gavage for 1 month. When the rats were 25 months old, they were sacrificed. The two knee joints were collected for histological description using toluidine blue staining. The tissues were classified according to the OARSI Score and stereological characteristics of the chondrocytes were determined.

**RESULTS:** The articular cartilage of the SR-EEP group was in the process of repair. The cartilage of the FC, M and TP presented a more organized appearance with an increase in thickness, articular surface and cationic staining of the matrix. The analysis with OARSI scale showed that there was a reduction in the degree of OA and a significant increase in the density of the chondrocytes compared to the SR and SR-V groups.

**CONCLUSION:** Histological and morpho-quantitative analysis of senescent knee articular cartilage suggests beneficial effects of EEP treatment *in vivo*, improving the OARSI score and increasing the number of chondrocytes present in the cartilage ( $p<0.005$ ).

Acknowledgements: DIUFRO N° DFP21-0041





### PP-019

#### Patrelina identification in *Apis mellifera iberiensis* through microsatellite markers

Sandra Martínez Ballano, Ester Sales, Pilar Santolaria, Jesús Yániz

BIOFITER research group, Environmental Sciences Institute (IUCA), Department of Animal Production and Food Sciences, University of Zaragoza, Huesca, Spain.

The only method that allows controlled breeding in the honey bee is artificial insemination, but apart from being costly, it is not as efficient as natural mating. An alternative is to develop controlled natural mating systems, but there is a need to have a tool to check whether the selected drones are actually those that mated with the queen. Molecular markers, in particular microsatellites, may be a useful tool for this purpose. The aim of this study was to validate microsatellite markers described in the literature for patriline identification in *A. m. iberiensis*. To address this objective, pupae samples from four colonies of *A. m. iberiensis* were sampled. Eight drone pupae (haploid) were collected in each hive to determine the queen genotype, and 24 worker pupae (diploid) to determine the paternal alleles, and thus for distinguishing paternal lines (patrilines) in each colony. Amplification of 9 microsatellite loci was tested, but 5 primer pairs did not produce the expected fragments or diversity, so finally 4 loci (A029, A107, A113 and Ap226) were used for colony genotyping. Markers A107 and A029 showed higher allelic diversity (13 and 10 alleles respectively), while only 3 and 4 alleles were observed for the A113 and Ap226 loci. This allelic variation allowed discriminating patrilines in the 4 hives (11, 12, 8 and 10, respectively), values that correspond to those obtained in previous studies, in which the subspecies *A. m. iberiensis* was not tested, and where the number of samples studied was much higher. The analyses also allowed to determine the genotype of the drones that had fertilized the queens in each hive. In conclusion, these 4 markers allowed the characterization of patrilines in *A. m. iberiensis* colonies, and can therefore be applied for paternity assessment in controlled crosses of this subspecies. This work was supported by the Spanish AEI-MICINN (grant PID2020-112673RB-100), and the DGA-FSE (grant A07\_17R).

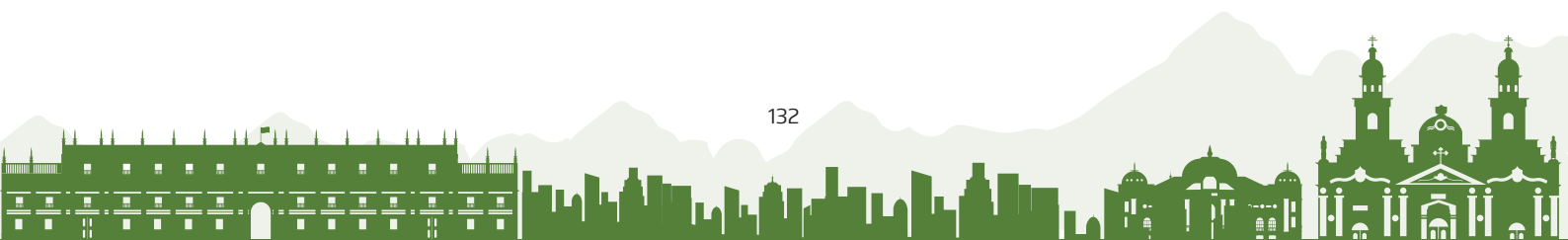
### PP-020

#### Population variability of bees and drones (Hymenoptera: Apidae) in a congregation area in the high Andean zone of Boyacá, Colombia

Guillermo Salamanca Grosso, Monica Osorio, Maria Jose Aroca Narváez

Mellitopalinalogical Research Group, Department of Chemistry, Faculty of Sciences. University of Tolima

The reproduction process of *Apis mellifera* L. (Hymenoptera: Apidae) occurs in defined congregation areas where natural mating occurs and males and unfertilized queen bees from different colonies converge. This work focused on the identification and characterization of a congregation drone zone in a biogeographic region of the low montane dry forest corridor, in the upper Andean valley of Sugamuxi-Tundama, for the density of bee colonies established there, considering the climatic factors of the environment and the description of biometric characters of bees and drones collected, and which constitutes the first report of a congregation zone for Colombia. In the study area, dry summer periods predominate alternating with short winter, cloudy days, and intense cold, with thermal sensations between 8 to 19°C. The drone movement route was established with an unmanned flight vehicle, through the interaction between the vehicle and bee specimens. The path of movement of drones was established operated between 10 and 60 m in North-South direction. Specimens of worker bees were collected identifying African, European, or Eastern European lines and drones of two types, yellow and black, evaluating biometric parameters and estimating the discriminant factor. The activity of the drones, occurs in two periods of 10:00 to 12:00 hours and 15:00 to 17:00 hours, flying regularly between round trips up to a 1 km radius. This work describes a methodology for monitoring congregation areas and consolidates itself as a tool for beekeepers for the conservation of regional ecotypes through bee reproductive planning



### PP-021

#### Requeening queenright honey bee colonies with queen cells in honey supers

Shelley Hoover<sup>1</sup>, Leslie Holmes<sup>1</sup>, Jeffery Kearns<sup>1</sup>, Lynae Ovinge<sup>3</sup>, Patricia Wolf Veiga<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, University of Lethbridge, Lethbridge, Canada

<sup>2</sup>National Bee Diagnostics Centre, Northwestern Polytechnic, Beaverlodge, Canada

<sup>3</sup>Alberta Beekeepers Commission, Edmonton, Canada

Canadian beekeepers replace a subset of their honey bee queens annually, however, the process of introducing a new queen to a honey bee colony comes with with no guarantee of success. Despite the consensus that it is most effective to introduce queens to queenless colonies, some commercial beekeepers introduce queen cells into the honey super of queenright colonies. We tested the success rate of this practice by introducing queen cells to 100 queenright colonies in southern Alberta during a honey flow. The maternity of the resultant offspring drones was determined using mitochondrial DNA to identify laying queens. Our results show that new queens successfully superseded the original queen in only 6% of queenright colonies, suggesting that the practice does not result in the new queen taking over leadership in the majority of colonies. Additionally, our results show that supersedure by daughter queens is more common (13%) than new queen supersedure when introducing queen cells to queenright colonies during a honey flow. However, there could be a benefit to the practice of requeening queenright colonies with queen cells in honey supers if the colonies that accepted a new queen (whether a daughter of or unrelated to the old queen) were colonies with a failing queen.

### PP-022

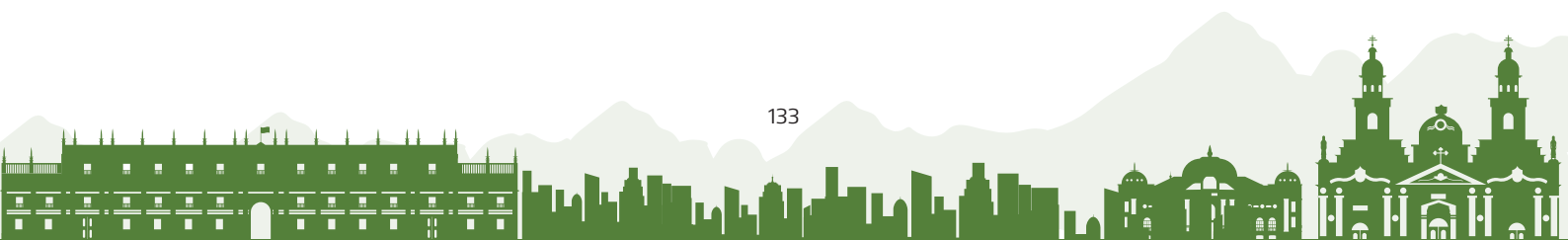
#### Modulation of protein and energy diets on consumption and their effect on immune cell population and survival of *Apis mellifera* worker caste

Karin Gloria Barrientos<sup>1</sup>, Roberto Carrillo<sup>2</sup>, Leticia Elena Silvestre<sup>2</sup>

<sup>1</sup>Escuela de Graduados, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile.

<sup>2</sup>Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile.

Honeybee (*Apis mellifera*) is a key economic and ecosystem insect. This species feeds naturally on pollen and nectar to obtain protein and carbohydrates respectively; but is also fed artificially by beekeepers. Worker bees are responsible for maintaining the flow of nutrients in the colony. As a result, they are constantly exposed to various etiological agents. To deal with them they have a cellular immune response that is mediated by hemocytes. These “blood cells” need a constant supply of food to maintain themselves and generate a response, but the consumption of these food sources does not affect the insect's survival. In the honeybee, the limited background on the relationship between the consumption of different types of diet and hemocytes is inconclusive and scarce. For this reason, the objective of this study was to determine the modulating effects of different pollen (high protein - low protein) and energy (honey - high fructose corn syrup HFCS) diets on the consumption rate and their impact on hemocyte concentration (THC) and survival of young worker bees. The results show that the daily consumption of the pollen diets was differential from 4-day-old bees, with the high-protein pollen diet having the highest daily consumption in 5- to 10-day-old worker bees. Consumption of the honey-based energy diet was significantly higher from 2 days of emergence compared to HFCS. THC was higher in the honey-based energy diet and lower in the low-protein pollen diet. Worker survival was not affected by the diets supplied. This research is the first to address the effect of high fructose corn syrup consumption on components of the cellular immune response.



### PP-023

## Use of different commercial formulations of pollen and honey substitutes to promote population growth of *Apis mellifera jemenitica* beehives in Fujairah, UAE

[James Salome](#), Shaher Bano, Fouad Lamghari Ridouane, Khawla Mohammed Alyammahi  
Fujairah Research Centre

Like all other animals, honey bees require essential ingredients for survival and reproduction. Honey bees require carbohydrates (sugars in nectar or honey), amino acids (protein from pollen), lipids (fatty acids, sterols), vitamins, minerals (salts), and water. Different formulations are produced for feeding the bees, mainly replacing honey and pollen in protein and sugar patty, which are applied directly on the brood frames in the hive nests. This study aims to evaluate the effect of commercial formulations of pollen and honey substitutes directly on the growth of the adult bee population in the tested hives. The experiment occurred in a commercial apiary in the emirate of Fujairah - UAE, between 02/21/2023 - 03/25/2023. Twenty *Apis mellifera jemenitica* beehives were separated into four lots of five hives each. Three lots received commercial protein patty feed (1 kg each) on 02/21/2023 and 03/13/2023. The first lot - T1- received Apipasta Plus, produced by Zukan, S.L.U. - Spain. The second batch - T2- received Activator, produced by Super Bee - Oman. The third batch - T3 - received Super Activator, produced by Super Bee - Oman. The fourth batch - T4 - was the Control, not receiving any treatment. The five hives of each of the four treatments were evaluated for population growth according to the coverage of frames with adult bees on 02/21/2023; 03/03/2023; 03/13/2023; 03/25/2023.

T1 showed better efficiency (100%) in promoting the population growth of the hives. In 33 days, starting with only five frames covered with adult bees (02/21/2023), it showed complete coverage of standard Langstroth hives with ten frames covered with adult bees on both faces on the day of the last evaluation (03/25/2023). The smallest population growth was in T4, which presented a value of only 4.2%, starting with 4.8 frames covered with adult bees (02/21/2023) and ending the experiment with five covered frames (03/25/2023). T2 and T3 showed intermediate growth values of 70% and 21%, respectively.

Apipasta Plus can be used about 30 days before the start of the honey production season as a pre-season beehive development tool.

### PP-024

## Effects of local domestication warrant attention in honey bee population genetics

Zheguang Lin<sup>1</sup>, Zhongxu Zhu<sup>2</sup>, Mingliang Zhuang<sup>3</sup>, Zhi Wang<sup>3</sup>, Yi Zhang<sup>1</sup>, Fuchao Gao<sup>4</sup>, Qingsheng Niu<sup>3</sup>, Ting Ji<sup>1</sup>

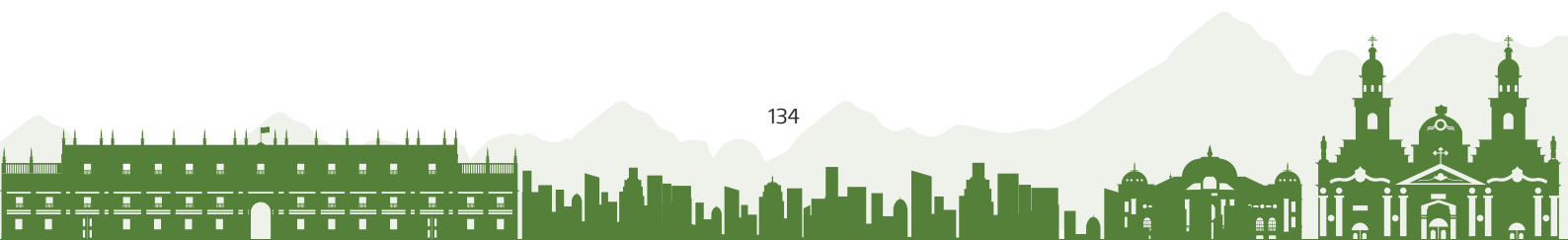
<sup>1</sup>Apicultural Research Institute, College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China

<sup>2</sup>Department of Biomedical Sciences, City University of Hong Kong, Hong Kong SAR, China

<sup>3</sup>Apiculture Science Institute of Jilin Province, Jilin 132108, China

<sup>4</sup>Mudanjiang Branch of Heilongjiang Academy of Agricultural Sciences, Mudanjiang 157043, China

Honey bees, *Apis mellifera*, have for millennia been managed and exploited by humans and introduced into most suitable regions worldwide. However, given the lack of records for many introduction events, treating *A. mellifera* populations as native would predictably bias genetic studies regarding origin and evolution. Herein, we used the Dongbei bee, a well-documented population, introduced beyond the natural distribution range approximately 100 years ago, to elucidate the effects of local domestication on animal population genetic analyses. Strong domestication pressure with a pronounced genetic drift was detected in this population, and the genetic divergence between Dongbei bee and its ancestral subspecies was found to have occurred at the lineage level. Notably, evolution over the documented ~100 years of local domestication is estimated to be equivalent to that expected over 2.5 million years of natural selection. The differing outcomes of phylogenetic analyses provide support that the composition of honey bee populations subjected to anthropogenic pressures could potentially bias evolutionary analyses. Considering that most of the *A. mellifera* colonies worldwide are under long-term management, particularly in the current post-Varroa destructor era characterized by few wild/feral populations, and that the generation time of honey bees is relatively short, the effects of local domestication warrant particular attention, and proposing new subspecies or lineages and performing analyses of origin should strive to eliminate anthropogenic effects. We highlight the need for definitions of landrace and breed in honey bee sciences.



### PP-025

#### Patrelina identification in *Apis mellifera iberiensis* through microsatellite markers

Sandra Martínez Ballano, Ester Sales, Pilar Santolaria, Jesús Yániz

BIOFITER research group, Environmental Sciences Institute (IUCA), Department of Animal Production and Food Sciences, University of Zaragoza, Huesca, Spain.

Given that queens and drones mate in free flights high in the air, the ability to control mating is one of the most challenging aspects of honeybee breeding programs. The only method that allows full controlled breeding in the honey bee is artificial insemination, but apart from being costly, it is not as efficient as natural mating. An alternative is to develop controlled natural mating stations and/or delayed mating systems, but there is a need to have a tool to check whether the selected drones are actually those that mated with the queen. Molecular markers, in particular microsatellites, may be a useful tool for this purpose. The aim of this study was to validate microsatellite markers described in the literature for patrilina identification in *A. m. iberiensis*. To address this objective, pupae samples from four colonies of *A. m. iberiensis* were sampled. Eight drone pupae (haploid) were collected in each hive to determine the queen genotype, and 24 worker pupae (diploid) to determine the paternal alleles, and thus for distinguishing paternal lines (patrilines) in each colony. Amplification of 9 microsatellite loci was tested, but 5 primer pairs did not produce the expected fragments or diversity, so finally 4 loci (A029, A107, A113 and Ap226) were used for colony genotyping. Markers A107 and A029 showed higher allelic diversity (13 and 10 alleles respectively), while only 3 and 4 alleles were observed for the A113 and Ap226 loci. This allelic variation allowed discriminating patrilines in the 4 hives (11, 12, 8 and 10, respectively), values that correspond to those obtained in previous studies, in which the subspecies *A. m. iberiensis* was not tested, and where the number of samples studied was much higher. The analyses also allowed to determine the genotype of the drones that had fertilized the queens in each hive. In conclusion, these 4 markers allowed the characterization of patrilines in *A. m. iberiensis* colonies, and can therefore be applied for paternity assessment in controlled crosses of this subspecies. This work was supported by the Spanish AEI-MICINN (grant PID2020-112673RB-I00) and the DGA-FSE (grant A07\_17R).

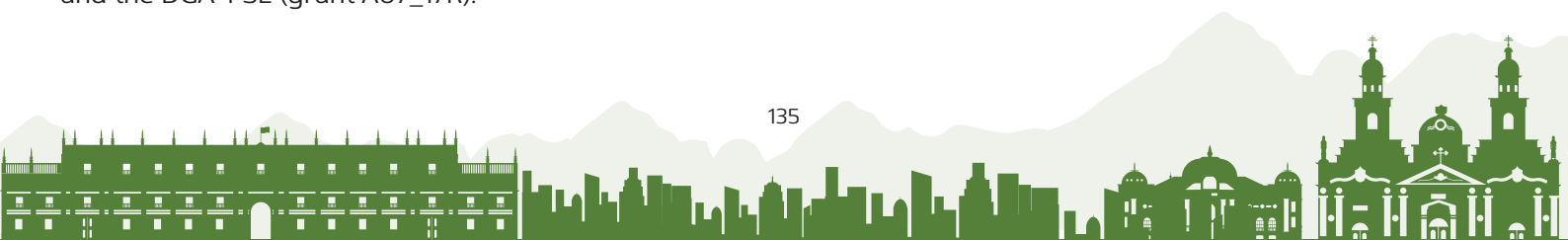
### PP-026

#### Wing morphometry subspecies differentiation in *Apis mellifera* using smartphone images and a free MATLAB-based tool

Ernesto Angel Beamonte, Pilar Santolaria, Susana Cortes Calvo, Jesús Yániz

BIOFITER research group, Environmental Sciences Institute (IUCA), Department of Animal Production and Food Sciences, University of Zaragoza, Huesca, Spain.

Wing morphometry has been found to be an invaluable tool for managing honey bee populations and promoting their conservation. In order to facilitate studies of honey bees, we present here a new software for the fully automatic and complete geometric morphometric analysis of the forewing venation structure used for identification of honey bee subspecies. This new software, called WingMarks2, requires a prior definition of a wing template, but all the images subsequently analyzed are automatically processed through formatting, orientation, and stylization, finally identifying the coordinates of each of the 19 landmarks that represent the wing vein union nodes using a spatial minutiae convolution algorithm. After landmark determination, WingMarks2 automatically performs a full Procrustes fit superimposition of the landmark coordinates and a Fisher's linear discrimination analysis to provide the results of subspecies classification. Two experiments were performed to evaluate the ability of WingMarks2 software to analyze wing images obtained with different optical equipment (experiment 1) to check its ability to discriminate between six subspecies of *Apis mellifera* (*A. m. iberiensis*, *A. m. mellifera*, *A. m. ligustica*, *A. m. carnica*, *A. m. intermissa*, and *A. m. sahariensis*) and between two evolutionary lineages within *A. m. iberiensis* (experiment 2). In experiment 1, the new software allowed the automatic and accurate precise analysis of 93.0%, 95.7% and 95.8% of the 645 wings analyzed when using Leica-microscope, Celestron USB-microscope and Xiaomi Smartphone images, respectively. Results of experiment 2 showed that, overall, the cross-validated correct identification rate of was 97.4 % for the subspecies as a whole and ranged between 94% and 100% for the individual subspecies. It was concluded that WingMarks2 is a powerful and efficient tool for the complete analysis of wing morphometry in *Apis mellifera* even using a smartphone camera for image acquisition. This work was supported by the Spanish AEI-MICINN (grant PID2020-112673RB-I00), and the DGA-FSE (grant A07\_17R).



### PP-027

## A key gene for the climatic adaptation of *Apis cerana* populations in China according to selective sweep analysis

Ting Ji<sup>1</sup>, Yi Zhang<sup>1</sup>, Xu Hao<sup>2</sup>, Zhi Wang<sup>3</sup>, Haoliang Jie<sup>4</sup>, Fuchao Gao<sup>5</sup>, Minqi Cai<sup>1</sup>, Kang Wang<sup>1</sup>, Dafu Chen<sup>6</sup>, Rui Guo<sup>6</sup>, Zheguang Lin<sup>1</sup>, Qingsheng Niu<sup>3</sup>

<sup>1</sup>College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China

<sup>2</sup>Sericultural Research Institute, Anhui Academy of Agricultural Science, Hefei 230061, China

<sup>3</sup>Apiculture Science Institute of Jilin Province, Jilin 132108, China

<sup>4</sup>Jinzhong Agriculture and Rural Affairs Bureau, Jinzhong 030601, China

<sup>5</sup>Mudanjiang Branch of Heilongjiang Academy of Agricultural Sciences, Mudanjiang 157043, China

<sup>6</sup>College of Animal Science (College of Bee Science), Fujian Agriculture and Forestry University, Fuzhou 350002, China

During the long-term natural evolutionary process, many unique phenotypic variations have occurred among *Apis cerana* populations in different geographical regions under varied climates. Understanding the molecular genetic basis and the effects of climate change on the adaptive evolution of *A. cerana* can promote *A. cerana* conservation in face of climate change and allow for the effective utilization of its genetic resources. We utilized population genetics methods and morphometric analysis to identify the key traces in the process of adaptation to different environments at the genome level. A total of 100 honeybee samples (covering the most important climate types) were studied in this experiment. The collection sites for each sample were centered in Suzhou, Jiangsu, and were located at similar latitudes and different longitudes or similar longitudes and different latitudes. In addition, 30 samples from sites with similar longitude and different latitude as Suzhou were included for comprehensive data analysis. Our results revealed an important relationship between climate types and the genetic variation of *A. cerana* in China, and a greater influence of latitude compared with longitude was observed. The population subdivision pattern classified by population genetic structure analysis and principal component analysis (PCA) showed similar results that JL, MK, and WC could be separated from the remaining closely related and difficult-to-separate populations. The JL, MK, and WC groups were highly differentiated compared with the other groups. Upon selection and geometric morphometry analyses combination for populations (JL, MK, WC, and SZ) under different climate types, we identified a key gene RPTOR, which was deeply involved in developmental processes and influenced the body size. The selection of RPTOR at the genomic level during adaptive evolution could allow *A. cerana* to actively regulate its metabolism, thereby fine-tuning body sizes in response to harsh conditions caused by climate change, such as food shortages and extreme temperatures, which may partially elucidate the size differences of *A. cerana* populations. This study provides crucial support for the molecular genetic basis of the expansion and evolution of naturally distributed honeybee populations.

### PP-028 [Bee Biology]

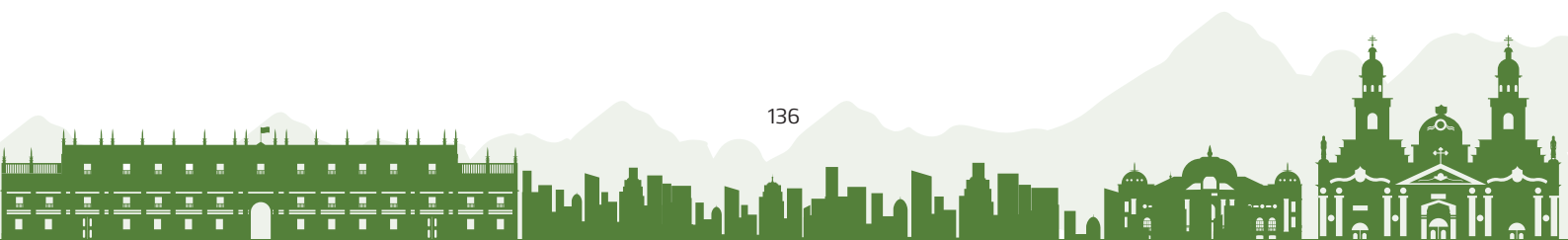
## The effect of abdominal exposure to 2, 4-dichlorophenoxyacetic acid on nursing and foraging capabilities of honey bees (*Apis mellifera* L.)

Hyun Jee Kim<sup>1</sup>, Yumi Yun<sup>2</sup>, Da In Lee<sup>2</sup>, Youn Sung Choi<sup>2</sup>, Olga Frunze<sup>1</sup>, Jeong Hyeon Lee<sup>2</sup>, Hyung Wook Kwon<sup>2</sup>

<sup>1</sup>Convergence Research Center for Insect Vectors, Incheon National University, Incheon, South Korea

<sup>2</sup>Department of Life Sciences, College of Life Science and Bioengineering, Incheon National University, Incheon, South Korea

The use of agrochemicals in agriculture has increased due to the global population and food demand. However, this increase has negative consequences for honeybees. 2, 4-dichlorophenoxyacetic acid (2, 4-D), a commonly used herbicide, destroys honeybees' natural habitat and causes direct poisoning, leading to colony collapse disorder. Yet, the effects of 2, 4-D on nursing and foraging behaviors related to sensory modulation in honeybees remain largely unknown. In this study, we investigated the effects of 2, 4-D on honeybees' nursing and foraging capabilities at the behavioral, molecular, and neurophysiological levels. We found that the exposure to 2, 4-D resulted in a significant reduction in the acini size and expression level of the umami taste receptor (AmGr10), which is involved in associative hypopharyngeal gland (HG) development. In addition, to identify the foraging capability effect, we demonstrated a significant reduction in the proboscis extension response to sugar taste, which is related to gustatory response, and Pavlovian conditioning related to olfactory responses. Moreover, we identified that the octopamine receptor, which is involved in associative olfactory learning and memory processes, was significantly decreased in the brain. The study provides strong evidence that 2, 4-D impairs nursing and foraging capabilities in honeybees, threatening pollination and agriculture.





### PP-029

#### Examining the effects of pollen substitute diets on honey bee colony performance after overwintering in South Korea

Hyun Jee Kim<sup>1</sup>, Myeong Lyeol Lee<sup>1</sup>, Jeong Hyeon Lee<sup>2</sup>, Yumi Yun<sup>2</sup>, Youn Sung Choi<sup>2</sup>, Da In Lee<sup>2</sup>, Olga Frunze<sup>1</sup>, Hyung Wook Kwon<sup>2</sup>

<sup>1</sup>Convergence Research Center for Insect Vectors, Incheon National University

<sup>2</sup>Department of Life Sciences, College of Life Science and Bioengineering, Incheon National University

Nutrition affects the health and strength of honey bee colonies. To evaluate the effects of different diets on the strength of honey bee colonies, seven types of diets and a control group (honey bees in their natural state without any diet) were prepared and tested in early spring in Gangneung, South Korea. Among the seven types of diets tested, two were commercial diets (Megabee and YBNH), and the other five were candidate pollen substitutes: Test A, AIGT+Soytide, AIGT+Apple juice, AIGT+Chlorella, and AIGT+Mint powder. A total of 27 honey bee colonies were used for 42 days to evaluate honey bee density, brood area, consumption, preference, head weight, vitellogenin (Vg) expression, and expression of deformed wing virus and sacbrood virus. In terms of honey bee density, brood area, consumption, and Vg expression, AIGT+Apple juice showed the highest statistically significant results. AIGT+Soytide had the highest preference. Overall, AIGT+Apple juice was found to be the most beneficial for maintaining and strengthening honey bee colonies and improving their health and productivity in early spring. Therefore, the results suggest that the candidate pollen substitute diets being developed could contribute to the healthy growth of honey bee colonies and help mitigate the decline in honeybee populations

### PP-030

#### Scientific-based evidence illustrating honey-bee meticulous care for a naturally self-regulated hive

Marie-agnes Courty<sup>1</sup>, Claude Grison<sup>2</sup>, Jean Michel Martinez<sup>1</sup>, Erwan Oliviero<sup>3</sup>, Franck Pelissier<sup>2</sup>, Rachel Pflieger<sup>4</sup>, Mariana Timm<sup>3</sup>, Catherine Flurin<sup>5</sup>, Boris Cabrol<sup>6</sup>

<sup>1</sup>PROMES UPR 8521 CNRS, Univ. Perpignan, Tecnosud, Perpignan, FR. marie-agnes.courty@promes.cnrs, France

<sup>2</sup>Chimeco, UMR 5021 CNRS, 1682 Rue de la Valsière, 34790 Grabels, France

<sup>3</sup>ICGM UMR 5253, Univ. Montpellier, 34095 Montpellier, France

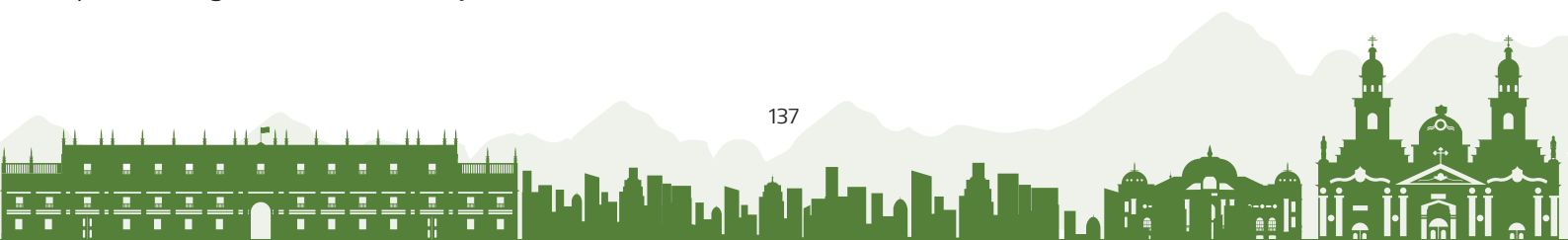
<sup>4</sup>ICSM UMR 5257 – CEA / CNRS / UM / ENSCM Site de Marcoule, Bagnols sur Cèze, France

<sup>5</sup>Société Abeilles Santé, 100 Rue des Moulins, 65700 Maubourguet, France

<sup>6</sup>Association Joseph-Sauvy, Chem. du Mas Taillant, 66430 Bompas, France

One of the major goal of the research project LINA - financially supported by the French Occitan region - was to investigate how honey-bees use the available resources for regulating the thermal and hydric equilibrium of the hive. This scope was achieved by focusing on polymeric nanocomposites that are produced at the venom ejection by honeybees from nanoparticule-rich resources. They are retrieved mainly in water, nectar, and pollens for the wax, honey and royal jelly production and in addition in plant exudates for the propolis making by honey-bees. At first, we present the analytical protocol that allowed to trace the in situ incorporation of venom solid-components to the different waxes, to honey, to royal jelly and to propolis along the successive hive-life stages. The venom injection by honey-bees is explained to incorporate within the hive droplets of energy resources, occurring as polymeric nanocomposites produced by plasma-discharge. Due to its exceptional mechanical, thermal, electrical and magnetic properties, this polymeric nanoassemblage is shown to favor the conductivity, the ductility and the microporosity of the waxes, the stability of the honey and of the royal jelly, and the malleability of the propolis. The comparison of different apiaries with diverse species allowed to observe the remarkable efficiency of the original black honey-bees for collecting the nanoconfined energy resources. The black honey-bees also appeared to be experts in the transformation of this polymeric assemblage by triboelectricity for the safe of hive products, particularly at the critical stage of honey sealing.

The comparison of Buckfast colonies bred on natural wax hives with the ones on plastic hives showed an interface between the plastic cells with the brood or with the honey cells of a microporous propolis-solid venom materials. This occurrence revealed the critical importance for the honey-bees to always have hive cells either for the brood or for honey storage made of alive organic materials. Their high content in nanoconfined energy is essential to the thermal and hydric regulation of the hive. The common trend to use plastic hives is thus concluded to severely constrain the optimal living conditions of honey-bees.



### PP-031

#### Hygienic Behavior Of Africanized *Apis Mellifera* Colonies In The Region Of Terenos-ms, Brazil

Matheus Portela Pinho<sup>1</sup>, Rodrigo Zaluski<sup>2</sup>, Diego Moure-Oliveira<sup>1</sup>, Joyce Almeida<sup>1</sup>, Carlos Pamplona Rehder<sup>1</sup>, Andresa Aparecida Berretta<sup>1</sup>

<sup>1</sup>AgroBee – Eleve Pesquisa & Desenvolvimento, Ribeirão Preto/SP;

<sup>2</sup>Universidade Federal do Mato Grosso do Sul, Campo Grande/MS;

The Africanized bees that originated in Brazil are the result of the crossbreeding of European and African subspecies. These bees generally exhibit a high hygienic behavior, which facilitates the removal of sick or parasite-infested brood, preventing the spread of diseases in the colony. The objective of this study is to evaluate possible seasonal changes in hygienic behavior (HB) and population development (PD) of Africanized honey bee colonies maintained in Terenos, Mato Grosso do Sul, Brazil, from 2020 to 2021. The experiment was performed with 10 hives, standardized in terms of queen age, adult bees population, and brood. The pin-killed brood assay was used to evaluate HB, and the removal of killed brood was evaluated after 24 hours. For PD evaluation, two brood frames were photographed within a gridded support to assess capped brood areas; the amount of food frames and frames occupied by bees were also quantified monthly. Environmental variables were obtained from the Campo Grande Meteorological Station. Data obtained for the analysis of HB were analyzed using the Kruskal-Wallis test, and colonies that showed more than 80% removal of pin-killed brood were classified as highly hygienic, and those below this percentage were classified as non-hygienic. Of the hives used, five were classified as hygienic, as they showed a removal rate of pin-killed brood between 82.25% and 93.07%; and five were classified as non-hygienic, as they showed between 62.67% and 79.06% removal of punctured brood. No seasonal changes were detected in the hygienic behavior of the evaluated colonies. The amount of capped brood, food frames, and frames occupied by bees showed no seasonal changes or differences between hives with high and low HB. During the study, it was observed that the hygienic behavior of the evaluated honey bee colonies varied constantly, but there was no correlation with population development, seasonality, or environmental conditions. This suggests that hygienic behavior is primarily determined by bee genetics. The study highlights the need for colony selection and genetic improvement programs to maintain health in beekeeping.

### PP-032

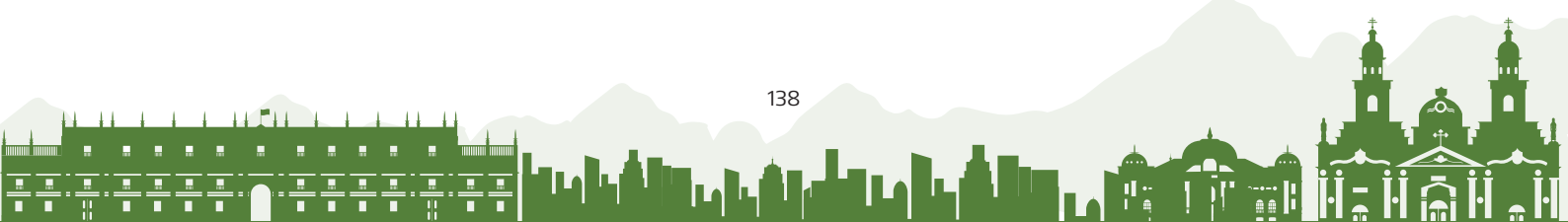
#### Evaluation of the effectiveness of liquid and solid energy diets in promoting the growth of Africanized colonies of *Apis mellifera* during the off-season in Brazil

Matheus Portela Pinho<sup>1</sup>, Rodrigo Zaluski<sup>2</sup>, Diego Moure Moure-Oliveira<sup>1</sup>, Joyce Almeida Dias<sup>1</sup>, Carlos Pamplona Rehder<sup>1</sup>, Andresa Aparecida Berretta<sup>1</sup>

<sup>1</sup>AgroBee – Eleve Pesquisa & Desenvolvimento, Ribeirão Preto/SP;

<sup>2</sup>Universidade Federal do Mato Grosso do Sul, Campo Grande/MS;

To reduce colony loss during times of low resource availability, it's essential to implement effective beekeeping practices. In order to evaluate the impact of various energy diets during the off-season period, a study was conducted between May and July 2020 at the Experimental Apiary of Universidade Federal de Mato Grosso do Sul, located in Terenos – MS– Brazil, using Africanized *Apis mellifera* colonies. Twenty swarms of Africanized bees kept in Langstroth hives were used. The colonies were standardized fifteen days before starting the experiment, so that they present the same amount of brood and food frames. Were evaluated two solid diets: commercial crystal sugar and Very High Polarizate sugar (VHP) (supplied in amount of 250 grams/colony twice a week, using a cover feeder); and two liquid diets: conventional sugar syrup and invert sugar syrup (with 50% sugar concentration supplied in a Boardman feeder, twice a week in the amount of 500 mL per colony). The amount of diets consumed by each colony was evaluated weekly. The mean amount of sugar consumption by colony was: 3,500; 4,188; 4,250 and 4,250 g in colonies that received commercial crystal sugar, VHP sugar, inverted sugar syrup, and conventional sugar syrup, respectively. There was lower consumption of commercial crystal sugar, however, without statistical difference ( $p > 0.05$ ). Total mean number of honeycombs constructed by colonies was 5.5; 6.2; 6.2; 6.0 and 3.5; and the final weight of colonies in July was 33.85; 38.30; 33.55; 33.70; and 26.70 kg in colonies that received commercial crystal sugar, VHP sugar, inverted sugar syrup; conventional sugar syrup, and colonies that did not receive any food, respectively. The number of honeycombs constructed and final weight of colonies that did not receive any food was lower ( $p < 0,05$ ) when compared to colonies that received artificial energetic diets. In this study, there was no difference regarding the supply of liquid and solid diets. The energetic supplementation of bee in the off-season minimized the effects of nectar scarcity and allowed the maintenance and development of colonies, preparing the colonies for the production period.



### PP-033

#### Hygienic behavior of *Apis mellifera* L. hives on dead brood of workers and drones

Amelia García Bernal, Jorge Luis Sanabria Cruz, Walberto Lóriga Peña, Jorge Demedio Lorenzo

Facultad de Medicina Veterinaria, Universidad Agraria de La Habana (UNAH), Cuba

Hygienic behavior (HB) is a primary mechanism of natural resistance of bees against brood diseases caused by bacteria, fungi and parasites. Although it has a genetic basis, it is known that there are external and intrinsic factors that influence its expression. The selection of bees according to the performance in this trait constitutes an effective measure for the improvement of resistance against the parasitic mite *Varroa destructor* and other pathogenic entities. Its evaluation has been carried out mainly in worker brood, but little studied in drone brood. In order to evaluate the expression of this mechanism on brood of both breeds, work was done in three apiaries in the province of Pinar del Río. The pin killed brood method with reading at 24 hours was used and 15 random selected hives were evaluated in each apiary, with three repetitions ( $n = 45 / \text{apiary}$ ). The hygienic behavior values were processed by comparison of proportions, between apiaries, samples and types of brood. The hives of the three apiaries showed a high hygienic behavior towards both types of brood, and although in worker brood it fluctuated from 0.00 % to 100.00 %, only four colonies did not show high HB, while the drone brood showed 78.00 % - 100.00 % of total removal.

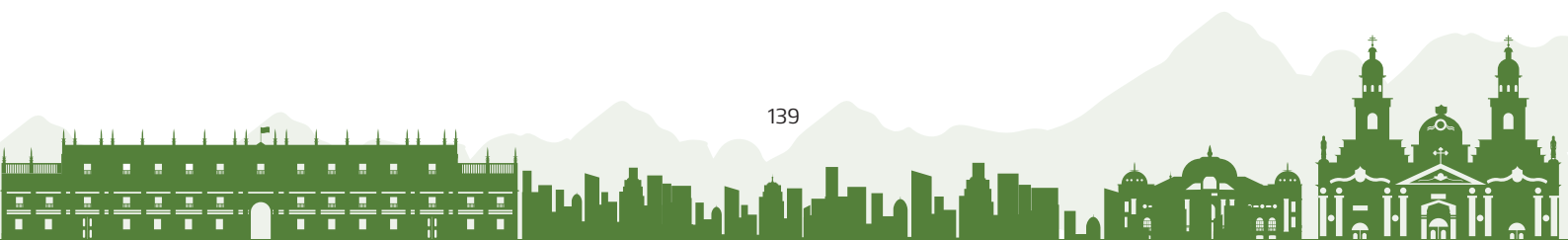
### PP-034

#### The quality of drones' semen stored under freezing conditions

Malgorzata Bienkowska, Mikolaj Boranski

Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

The aim of the study was to evaluate the quality of honey bee drones' semen in various storage periods in vitro and in vivo. Collected semen was stored in 24 capillaries of 50  $\mu\text{l}$  at  $14 \pm 1^\circ\text{C}$  for 3, 6 and 12 weeks. Semen quality control was conducted: a) immediately after semen collection to determine its original quality, b) after storage in the temperature of  $14 \pm 1^\circ\text{C}$  in different periods. In addition, young bee queens at 7 days of age were inseminated with a single dose of 8  $\mu\text{l}$  semen. For each group of fresh and stored for 3, 6 and 12 weeks semen 10 queens were inseminated. To assess the quality of the insemination, the oviducts and spermathecae of the inseminated queens were dissected after 72 hours. Sperm counts in semen from capillaries and spermathecae, were evaluated in 25 Bürker counting chambers. The ratio of live to dead sperm was assessed using the Live/Dead Sperm Viability Kit (Invitrogen™ by ThermoFisher Scientific), consisting of SYBR-14 and propidium iodide, and sperm motility was observed under a phase-contrast microscope. The results indicate that semen progressively degrades and the quality of bee queens inseminated with all groups of stored semen were worse in comparison to queens inseminated with fresh semen. However, the number of sperm in the spermathecae of queens inseminated with stored semen for 3 and 6 weeks, reaching 3 million, is satisfactory considering that in Polish breeding practice long and abundant egg laying of queens is not required. Research project entitled "Storage of semen of honey bee drones a guarantee of maintaining the gene pool of bees with the highest breeding and utility value" funded by Polish Ministry of Education and Science, CONTRACT No. MEiN/2022/DPI/181319 August 2022.



### PP-034

#### The quality of drones' semen stored under freezing conditions

Malgorzata Bienkowska, Mikolaj Boranski

Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

The aim of the study was to evaluate the quality of honey bee drones' semen in various storage periods in vitro and in vivo. Collected semen was stored in 24 capillaries of 50 µl at 14±1°C for 3, 6 and 12 weeks. Semen quality control was conducted: a) immediately after semen collection to determine its original quality, b) after storage in the temperature of 14±1°C in different periods. In addition, young bee queens at 7 days of age were inseminated with a single dose of 8 µl semen. For each group of fresh and stored for 3, 6 and 12 weeks semen 10 queens were inseminated. To assess the quality of the insemination, the oviducts and spermathecae of the inseminated queens were dissected after 72 hours. Sperm counts in semen from capillaries and spermathecae, were evaluated in 25 Bürker counting chambers. The ratio of live to dead sperm was assessed using the Live/Dead Sperm Viability Kit (Invitrogen™ by ThermoFisher Scientific), consisting of SYBR-14 and propidium iodide, and sperm motility was observed under a phase-contrast microscope. The results indicate that semen progressively degrades and the quality of bee queens inseminated with all groups of stored semen were worse in comparison to queens inseminated with fresh semen. However, the number of sperm in the spermathecae of queens inseminated with stored semen for 3 and 6 weeks, reaching 3 million, is satisfactory considering that in Polish breeding practice long and abundant egg laying of queens is not required. Research project entitled "Storage of semen of honey bee drones a guarantee of maintaining the gene pool of bees with the highest breeding and utility value" funded by Polish Ministry of Education and Science, CONTRACT No. MEiN/2022/DPI/181319 August 2022.

### PP-035

#### Breeding activities and mating control in three Balkan countries

Marin Kovačić<sup>1</sup>, Zlatko Puškadija<sup>2</sup>, Josipa Štavalj<sup>2</sup>, Sreten Andonov<sup>3</sup>, Bjørn Dahle<sup>4</sup>, Borce Pavlov<sup>5</sup>, Aleksandar Uzunov<sup>6</sup>, Janez Prešern<sup>7</sup>

<sup>1</sup>Faculty of Agrobiotechnical Sciences Osijek, University of Osijek, Osijek, Croatia

<sup>2</sup>Centre for applied life sciences Healthy Food chain Ltd (CALIS), Osijek, Croatia

<sup>3</sup>Swedish University of Agricultural Sciences, Department of animal breeding and genetics, Uppsala, Sweden

<sup>4</sup>Norwegian Beekeepers Association, Kløfta, Norway

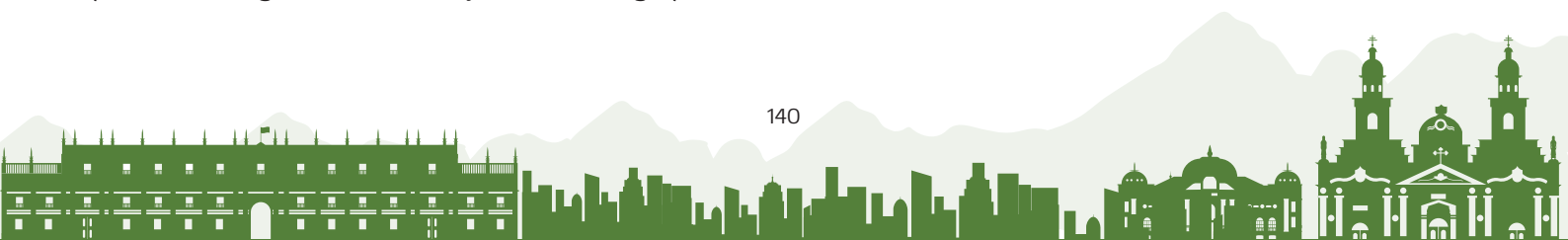
<sup>5</sup>Company for Applied Research and Permanent Education in Agriculture, Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Skopje, Macedonia

<sup>6</sup>University Ss. Cyril and Methodius in Skopje, Institute of Agriculture, Skopje, Republic of Macedonia

<sup>7</sup>Agricultural Institute of Slovenia, Ljubljana, Slovenia

The main objective of this study was to evaluate the state of the art of honey bee breeding activities and mating control as a key tool for both conservation and selection in three countries: Croatia (HR), Slovenia (SI) and Macedonia (MK). A survey was conducted on a total of 88 honey bee breeders: 36 from HR, 31 from SI and 21 from MK which covers more than 70 % of registered breeders in these three countries. The aim of the survey was to assess the current status of queen production, breeding and mating control in the operations. The survey consisted of 59 questions related to general information (15 questions), breeding and queen rearing (23 questions) and mating control (21 questions).

Based on survey results we conclude that breeders have knowledge and skills in technical aspects of queen production, which is shown by the high success of grafting, queen hatching and mating which they achieve. There seemed to be excellent foundations for introduction of controlled mating: breeders are well organized in breeders' associations; they have knowledge and skills for queen production and developed system for colony performance evaluation and selection. On top of that, survey showed that great majority of breeders are aware that breeding honey bees adapted to the local environmental conditions is very important and that the controlled mating is one of the major requirements to achieve genetic improvement of stock. However, the survey also revealed that one of the main challenges in selection progress is the lack of mating control: less than 0.1% of queen bees produced in the three countries are full pedigree queens (mated under strict mating control) which may be partly a consequence of much higher costs of controlled mating. Most of surveyed breeders will be in business for more than a decade, so it is worthwhile to invest time and money to facilitate mating control of queens in these countries. Additional awareness among the customers of the added value of buying full pedigree queens could also contribute to implementation of improved mating control in honey bee breeding operations.



### PP-036

#### Bionomic characteristics of *Scaptotrigona bipunctata* brood discs in two seasons of the year in the region of Maringá-Paraná-Brazil

Alessandra Benites<sup>1</sup>, Wendy Guadalin De Oliveira<sup>2</sup>, Daiani Rodrigues Moreira<sup>3</sup>, Isac deMacedo da Penha<sup>1</sup>, Vagner deAlencar Arnaut de Toledo<sup>4</sup>

<sup>1</sup>Postgraduate Program in Animal Science, State University of Maringa, Maringa, Brazil

<sup>2</sup>Postgraduate Program in Animal Science, Federal Technological University of Parana, Dois Vizinhos, Brazil

<sup>3</sup>Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, Maringá, Brazil

<sup>4</sup>Professor at the State University of Maringa, Animal Science Department and Postgraduate Program in Animal Science

Meliponiculture refers to the rational exploitation of stingless bees, in which different species are worked. What characterizes different morphology, biology, behavior and bionomic aspects according to the species. The study of such characteristics enables differentiation and comparison between species, or even variation within the same genus. The aim of the study was to verify whether different seasons influence the bionomic characteristics of *Scaptotrigona bipunctata* brood discs. The research was carried out at the Laboratory of Beekeeping and Meliponiculture, 16 colonies of *S. bipunctata* bees were evaluated, in the region of Maringá-Paraná-Brazil, in the year 2021 in the autumn and spring seasons. The collected data were brood disc (quantity, height, diameter, width) and brood cell diameter. Measurements were taken from the first three clearest discs (with older offspring) found in the colony. The brood cell diameter data were collected from four cells, these located at the top of the first disk on the left side. Measurements were performed using a digital caliper and a penknife for better handling and handling of the disks, in order to avoid damage and deterioration of the colony as a whole. The data were submitted to the t-test, with a significance level of 5%, to compare means between stations. The values found for total brood disc and total brood cells did not differ statistically between the autumn and spring seasons. The values of other characteristics such as disc height  $5.67 \pm 4.88$  and  $7.07 \pm 0.20$ , width  $87.77 \pm 1.56$  and  $77.12 \pm 2.02$ , length  $94.19 \pm 6.39$  and  $78.94 \pm 1.94$ , brood cells  $3.08 \pm 0.04$  and  $3.43 \pm 0.04$ , these measured in millimeters, differed significantly between seasons. It is concluded that the seasons of autumn and spring in the year 2021 influenced some bionomic characteristics of the brood discs for colonies of *S. bipunctata* in the region of Maringá-Paraná-Brazil.

### PP-037

#### Apparent digestibility in honeybee (*Apis mellifera* L.): titanium dioxide (TiO<sub>2</sub>) as an inert marker

Matías Francisco Ledesma González<sup>1</sup>, Leticia Andrea Fernández<sup>2</sup>, Elián Tourn<sup>3</sup>, Francisco José Reynaldi<sup>4</sup>

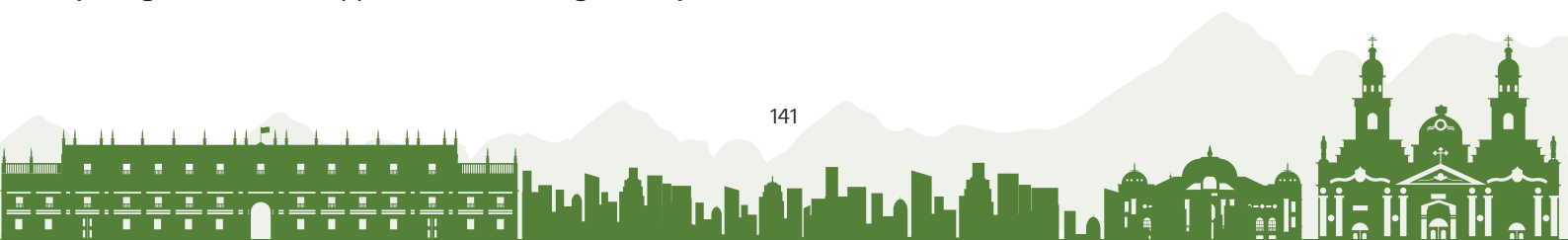
<sup>1</sup>Laboratorio de Estudios Apícolas (LabEA-CIC), Departamento de Agronomía, Universidad Nacional del Sur (UNS). Bahía Blanca, Prov. Buenos Aires, Argentina - Estudiante de doctorado, Becas Doctorales Temas Estratégicos CONICET

<sup>2</sup>Laboratorio de Estudios Apícolas (LabEA-CIC) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

<sup>3</sup>Laboratorio de Estudios Apícolas (LabEA-CIC) - Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC) - Cooperativa de Trabajo Apícola Pampero Ltda.

<sup>4</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Centro de Microbiología Básica y Aplicada (CEMIBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (UNLP). La Plata, Prov. Buenos Aires, Argentina

Digestibility refers to the extent to which feedstuff and its nutrients are absorbed in the digestive tract of an animal and utilized by the body. Apparent digestibility is a widely used method for measuring nutrient and feedstuff digestibility. This method requires an inert marker such as titanium dioxide (TiO<sub>2</sub>). TiO<sub>2</sub> had never been used in apparent digestibility studies in domestic honeybee. Thus, the aim of this work was to evaluate, for the first time, the behavior of TiO<sub>2</sub> as inert marker for apparent digestibility studies in *A. mellifera* under in vitro conditions. Two experiments were carried out: TiO<sub>2</sub> in sucrose syrup and TiO<sub>2</sub> in bee pollen, with five treatments each: 0 (control) - 0.1 - 0.25 - 0.5 - 0.75 % TiO<sub>2</sub> weight by weight based on dry mass basis. Eight replicates were used per treatment. A randomized complete block design was used with half of the replicates of both experiments on two dates (November 2021 and December 2021). We measured consumption variables (water, sucrose and pollen consumption and sucrose mobilization), apparent digestibility, mortality rate and body parts weight. The results revealed that sucrose consumption and mobilization, mortality rate and body parts weight did not differ between the different concentrations of inert marker used in both experiments. However, when concentration of TiO<sub>2</sub> increased, water consumption was reduced in both feeds provided. Apparent digestibility was 94,22% ( $\pm 2,2$ ) for sucrose syrup and 48,61 % ( $\pm 10,4$ ) for bee pollen. Like water consumption, apparent digestibility decreased as the inert marker concentration augmented. The use of TiO<sub>2</sub> as an inert marker for digestibility studies in *A. mellifera* is viable. The decline of water consumption and apparent digestibility with the increase of TiO<sub>2</sub> concentration in the feeds could be associated with a toxic effect on bee physiology. Inert marker concentration and apparent digestibility relation could also be explained to the analytical procedure. Based on these results, we selected a TiO<sub>2</sub> concentration of 0.1 % weight by weight for further apparent nutrient digestibility studies.





### PP-038

#### Honeybee colonies *Apis mellifera* L. perform orientation aggressiveness which varies among bred lines

Peter Njukang Akongte<sup>1</sup>, Bo Sun Park<sup>2</sup>, Dong Won Kim<sup>2</sup>, Yong Soo Choi<sup>2</sup>

<sup>1</sup>Department of Agricultural Biology, National Institute of Agricultural Science, Wanju, Republic of Korea, Department of entomology, Institute of Agricultural Research for Development, Buea, Republic of Cameroon

<sup>2</sup>Department of Agricultural Biology, National Institute of Agricultural Science, Wanju, Republic of Korea

Honeybees *Apis mellifera* L. are social organisms that express complex behavioral patterns (aggressiveness) to exhibit defensive mechanisms for their survival. The phenotypic expression of this behavior is influenced by both internal and external stimuli. Knowledge of honeybee orientation aggressiveness is recently being important. Practicing controlled breeding is a key to overcome the challenges of selecting bred lines. Chemical cues (alarm pheromone and isopentyl acetate mixed with paraffin oil), accompanied with physical and visual stimuli (5 by 5 cm dark leather suede, colony marbling and suede jiggling) were used to evaluate aggressiveness and orientation among five bred lines of honey bee colonies at 10sec, 30sec, 60sec, and 90sec. Both chemical assays recruited honeybees but the time of recruitment was significantly lower for alarm pheromone ( $t = 1.965$ ,  $P = 0.0004$ ). Honeybees response to both assays culminated to stings which differed among bred lines for alarm pheromone ( $K = 25.185$ ,  $df = 4$ ,  $P < 0.0001$ ) and paraffin ( $K = 14.551$ ,  $df = 4$ ,  $P = 0.006$ ) when colonies were marbled. Honeybees performed orientation aggressiveness which varied among bred lines and higher in more aggressive bred lines compared to less-aggressive bred lines. It is crucial to repeatedly evaluate orientation aggressiveness at colony level and among bred lines when selecting defensive and less-defensive honeybee colonies for breeding. Further studies should be conducted to evaluate the different concentrations of isopentyl acetate mixed with paraffin oil necessary to elicit orientation aggression in honeybee colonies within the shortest possible time.

### PP-039

#### *Apis mellifera* subspecies mitochondrial genome diversity

Leigh Boardman<sup>1</sup>, Rebecca T. Kimball<sup>2</sup>, Edward L. Braun<sup>2</sup>, Stefan Fuchs<sup>3</sup>, Bernd Grünewald<sup>3</sup>, James D. Ellis<sup>4</sup>

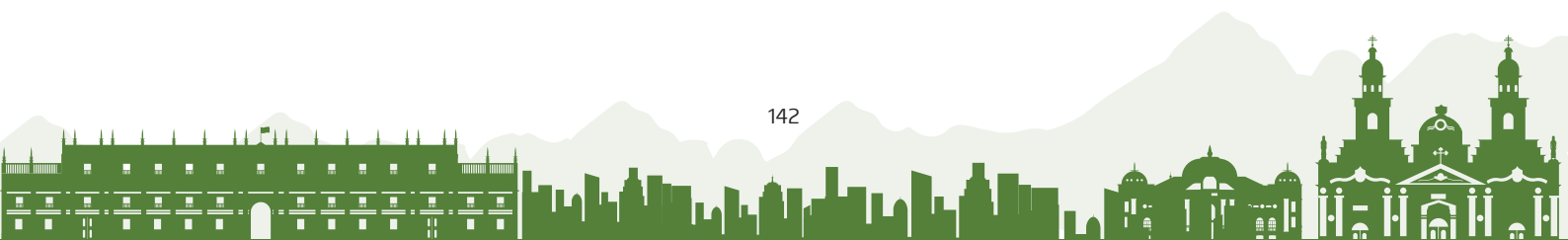
<sup>1</sup>Department of Biological Sciences & Center for Biodiversity Research, University of Memphis, Memphis, TN, USA

<sup>2</sup>Department of Biology, University of Florida, Gainesville, FL, USA

<sup>3</sup>Institut für Bienenkunde, Polytechnische Gesellschaft, Goethe-Universität Frankfurt am Main, Oberursel, Germany

<sup>4</sup>Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

With ~30 recognized subspecies spread throughout Africa, the Middle East, Europe and western Asia, and several breeds/strains introduced throughout the world, *Apis mellifera* Linnaeus 1758 (western honey bee) has a truly global distribution. Despite the economic importance of this species, molecular identification of different subspecies can be problematic in some regions - often due to ongoing hybridization, or a lack of reference sequences from understudied subspecies. While current methods are appropriate to identify honey bees to clades (often referred to as lineages), further identification of specific subspecies, breeds, or strains can be challenging. Using morphometrically confirmed samples representative of their subspecies (collected from central locations within ranges) from the Ruttner Bee Collection at the Bee Research Institute in Oberursel, Germany, we aimed to improve our understanding of the mitochondrial clades using broader representation of North African and African/Near East honey bees. Here, we present phylogenies of 91 mitochondrial genomes from our data and from GenBank and show where morphometric- and mitochondrial genome-based identifications are similar, and where they differ. This dataset has been used to identify mitochondrial gene regions for subspecies marker(s) and will highlight important aspects of *A. mellifera* biogeography for future studies.



### PP-040

#### Towards a molecular marker for defensive African-hybrid honey bees in the USA

Leigh Boardman<sup>1</sup>, Jose A. P. Marcelino<sup>2</sup>, Matthew R. Moore<sup>3</sup>, James C. Fulton<sup>3</sup>, Hector Urbina<sup>3</sup>, Laura Patterson Rosa<sup>4</sup>, James D. Ellis<sup>2</sup>

<sup>1</sup>Department of Biological Sciences and Centre for Biodiversity Research, The University of Memphis, Memphis, TN, USA

<sup>2</sup>Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

<sup>3</sup>Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL, USA

<sup>4</sup>Department of Agriculture and Industry, Sul Ross State University, Alpine, TX, USA

African-hybrid honey bees in the Americas often exhibit a more defensive phenotype, necessitating their identification for regulatory control. These bees originated in Brazil from the accidental release and subsequent hybridization of *Apis mellifera scutellata* Lepeletier (from southern Africa) with *A. m. ligustica* Spinola and *A. m. mellifera* Linnaeus (both from Europe). Now, they are widespread throughout South and Central America and the southern USA. Current molecular methods for their identification target mitochondrial COI-COII or cytochrome b (cytb) genes. However, these markers are not specific, and have a high risk of false positives for other African subspecies – including subspecies known to have been introduced in the USA, e.g., *A. m. intermissa* Buttel-Reepen. Here, we discuss our ongoing work to improve on the existing molecular detection methods. We have updated the original BglIII restriction enzyme assay that targeted a single nucleotide polymorphism (SNP) in cytb to function as a high-throughput qPCR assay. This assay can differentiate honey bees of African (A-lineage) ancestry from those of all other lineages, but it cannot identify specific subspecies within the A-lineage. The ongoing use of this assay by scientists from the Florida Department of Agriculture and Consumer Services (Gainesville, FL, USA) on samples from several defensive honey bee colonies in Florida allowed us to sequence cytb for additional candidate markers. Sequences revealed a SNP present in these defensive honey bees that is also found in GenBank archived data from honey bees sampled in Brazil, Mexico, South Africa, and the USA. This candidate SNP has been developed into a qPCR assay and applied to over 100 samples. While not a definitive marker for all defensive African-hybrid bees in the USA, it does provide increased accuracy for defensive honey bees in Florida, and warrants further investigation.

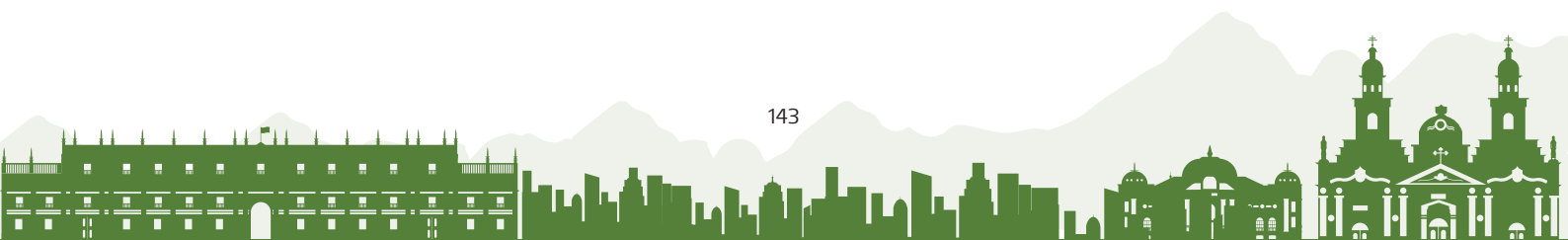
### PP-041

#### Breeding of *Apis cerana koreana* honey bees for honey production compared to the traditional collection method and *Acfor* gene expression

Yong Soo Choi, Dong Won Kim, Su Bae Kim, Bo Sun Park, Akongte N. Peter, Min Ung Son, Dae Geun Oh, Chang Hun Lee, You Young Jo

Department of Agricultural Biology, National Institute of Agricultural Science, RDA, Wanju, 55365 Republic of Korea

Improving honey yield is a crucial concern in global beekeeping. Typically, honey extraction is used simultaneously to collect honey and assess honey bees, but this can vary from year to year, which has led to the development of alternative selection methods. The aim of this study was to analyze honey-related traits in order to update honey bee pre-selection methods. *Apis cerana koreana* X, R lines, RX crossbred were evaluated using traditional (Weekly honey control and Cut-honey) methods, internal morphological traits (Load mass10, Load mass30, Load massAll, Maximal Load massAll, Load (%) and Nectar crop weight), and *Acfor* gene expression. X-line colonies were selected for significantly higher ( $P < 0.0001$ ) Load mass10, and Load mass30 which had high discriminatory power ( $P < 0.0001$ ) and low positive correlation to traditional methods. Our observations can be used for improving the method of honey bee selection for honey production and reducing dependence on nectar flow. However, further testing on *Apis mellifera* honey bees is necessary to confirm the universality of these criteria.



### PP-042

#### Cumulative food consumption, in the longevity and expression of the DWV, VG and MRJP1 genes, in *Apis mellifera* bees in the laboratory

José María Tapia González<sup>1</sup>, Jose Carlos Tapia Rivera<sup>2</sup>, Miguel Corona Villegas<sup>3</sup>, Jose Octavio Macias-Macias<sup>4</sup>, Francisca Contreras Escareño<sup>5</sup>, Ernesto Guzman-Novoa<sup>6</sup>

<sup>1</sup>José M Tapia-González Departamento de Cs. de la Naturaleza/CUSur/Universidad de Guadalajara/México

<sup>2</sup>José C Tapia-Rivera Departamento de /CUSur/Universidad de Guadalajara/México

<sup>3</sup>Miguel Corona-Villegas Laboratorio de Abejas/Departamento de Agricultura EEUU

<sup>4</sup>José O Macias-Macias Departamento de Cs. de la Naturaleza/CUSur/universidad de Guadalajara, México

<sup>5</sup>Francisca Contreras-Escareño Departamento de producción Agrícola/CUCSur/Universidad de Guadalajara

<sup>6</sup>Ernesto Guzmán-Novoa/Centro de Investigación en Abejas/Universidad de Guelph/Ontario Canadá

The lack of nutrients in the diet of honeybee colonies (*Apis mellifera*) can cause nutritional stress, being one of the main causes of the deterioration of the immune system and susceptibility to diseases. The aim of this work was to evaluate the accumulated consumption (CA) of Apitir<sup>®</sup>, Ultrabee<sup>®</sup>, and USDA Diet in the longevity and expression of the genes DWV, VG, and MRJP1, in *Apis mellifera* in the laboratory. Hybrid bees between one and two days of emergence were used. PCR of the genes DWV, VG, and MRJP1 of the bees was performed once a week until the end of the experiment, VG was also measured in bees within two days of emergence. The CA was evaluated by one-way ANOVA and Bonferroni. Longevity was represented by survival curves. Survival was analyzed by (ANOVA) of Bonferroni. Vg and mrjp1 were analyzed by one-way ANOVA and Bonferroni. The analysis of DWV was performed by Mann Whitney U. Gene expression and viral titers was evaluated by Spearman correlation. The highest CA for the bees was for the USDA Diet, (21 days), with 0.36 g. There were differences between the consumption of Apitir<sup>®</sup> and the USDA Diet with a value of  $Q = 5.19$  ( $p = .00128$ ). Longevity was higher for Ultrabee, there were differences between the longevity of Apitir<sup>®</sup> and Ultrabee<sup>®</sup>  $Q = 4.96$  ( $p = 0.00206$ ). The ANOVA did not show differences between treatments for VG. The USDA Diet quantified 972 copies for DWV, followed by Apitir<sup>®</sup> with 58 copies, which were statistically different, Ultrabee<sup>®</sup> and USDA Diet  $U = 0$  ( $p = 0.04953$ ). The USDA Diet presented higher levels of mRNA expression for mrjp1, followed by the levels of newly emerged bees, the ANOVA presented differences between Apitir<sup>®</sup> and USDA Diet groups  $F = 11.92218$  ( $p = 0.0259$ ). The bees in this study showed statistical differences in the accumulated consumption of protein foods of treatments, which influenced longevity, DWV loads and mRNA levels, but not in the expression of Vg. Key words: *Apis mellifera*, alimentos proteicos, consumo acumulado, DWV, VG y MRJP1

### PP-043

#### Differences in the semen quality of *Apis mellifera* drones of selected lines along reproductive season in Balcarce district, Southeastern Buenos Aires Province, Argentina

Facundo René Meroi Arcerito<sup>1</sup>, Analía Martínez<sup>3</sup>, Belén Bedascarrasbure<sup>2</sup>, Juan Lobo<sup>3</sup>, Federico Hozbor<sup>2</sup>, Alejandra Palacio<sup>2</sup>

<sup>1</sup>Social Bee Research Center (CIAS), Institute for Research in Production, Health and Environment (IIPROSAM), Faculty of Exact and Natural Sciences, National University of Mar del Plata (UNMdP), Mar del Plata, Argentina. Institute for Research in Health, Production, and Environment, National Council for Scientific and Technical Research (IIPROSAM-CONICET)

<sup>2</sup>National Institute of Agricultural Technology (INTA). Institute of Innovation for Agricultural Production and Sustainable Development (IPADS-Balcarce INTA.CONICET)

<sup>3</sup>Faculty of Agricultural Sciences, National University of Mar del Plata (FCA-UNMdP). Buenos Aires, Argentina.

Sperm quality in *Apis mellifera* drones plays a central role in colony reproduction. Several authors claimed that semen quality varies according to intrinsic and extrinsic factors. However, paramount and basic issues are unresolved in disentangling the factors affecting sperm quality. Here we explored if different dates within the reproductive season exhibit variation in sperm quality of drones from colonies selected under a template climate. Drones come from colonies of the Honeybee genetic program (MEGA-INTA), where lineages of brood disease tolerances are reared along with other features sought by the apiculture sector. The essay consists of three sampling times (T1, T2, T3) involving the whole reproductive season (from November to February), where T1 represents the beginning, T2 the middle, and T3 the ending reproductive season. Eight hives were assessed twice per sampling time. The techniques used were Eosina-Nigrosina for viability, Osmotic Swelling test (HOST) to test cell membrane function by using distilled water 100 OSM at 22°C and 35°C, concentration (number of spermatic cells estimation per volume of semen) through a Neubauer chamber, the number of drones matures with semen (total drones evaluated/drones with ejection of semen under abdomen stimulation), and the obtained sperm volume per drone (obtained volume/number of drone with semen) per colony. Results suggest T2 has better viability than T1 and T3 (Mann-Whitney:  $p = 0,00189$  and  $p = 0,005264$ , respectively). In HOST with distilled water, T2 differs from T3 (Mann-Whitney:  $p = 0,01344$ ). At 100 OSM at 22°C, there were no differences (all  $P > 0.05$ ), and at 100 OSM at 35°C, we only registered differences between T2 and T3 (Mann-Whitney:  $p = 0,01639$ ). In Concentration, T2 had more sperm cells than T1 and T3 (Mann-Whitney:  $p = 1,05E-05$  and  $p = 2,25E-06$ , respectively). In semen volume per drone and the number of mature drones with semen, there were no differences (all  $P > 0.05$ ). Our results suggest variation in semen quality through the reproductive season. Hence, in the sampling region, we recommend using reproductive techniques involving semen manipulation, preferably in January (T2), involving better quality semen.



### PP-044

#### Effect of the protein supplement PROSISA® on the posture and population of commercial hives during pollen harvest

Yhann Vallenás-Sánchez<sup>1</sup>, Laura Yazmin Blas Cortijo<sup>2</sup>, Deysi Yenny Ruiz Llontop<sup>2</sup>, Juan Carlos Rodríguez-Soto<sup>1</sup>

<sup>1</sup>CAPDES, Department of Agronomy and Zootechnics, National University of Trujillo, Trujillo, Perú.

<sup>2</sup>Universidad Privada Antenor Orrego, Trujillo, Perú

The honey bee (*Apis mellifera* L.) plays an important role in the pollination of crops destined for human consumption. However, climate change, monocultures, and deforestation reduce the quantity and variety of pollen. This reduces queen bee oviposition, the life span of the bees, the bee population, and the productive performance of the colonies. For this reason, artificial diets are used to reduce the negative impact of protein shortage. The objective of the present study was to evaluate the effect of the protein supplement PROSISA-P® on the posture and population of commercial pollen trap hives. The PROSISA® protein supplement was developed using Animal-Space® software and the supplement inputs were: brewer's yeast, whey, vitamin and mineral premix, probiotics, pollen, egg albumin, L-isoleucine, L-threonine, L-methionine, L-valine, L-tryptophan, sugar syrup and corn oil. For the field study, 12 commercial hives installed in poly floral crops (charamusco, eucalyptus, and corn) were chosen. The field study was carried out in Casique-La Libertad-Peru, during the month of December 2022 (24 days). The colonies received two treatments (T0: without protein supplement and T1: with 250g/15 days PROSISA® protein supplement). Pollen traps were used in all hives. Our results showed that PROSISA® significantly increased the number of frames with brood (7.0 frames, p-value=0.0359) and frames populated with bees (12.83 frames, p-value=0.0001) with respect to the control treatment colonies (5.5 and 7.7 frames respectively). We conclude that the use of the PROSISA® protein supplement increased the laying and population of commercial hives in pollen harvest. We recommend evaluating different periods of pollen harvest and the effect of different protein levels on posture and hive population.

### PP-045

#### Addressing the knowledge gap on bee diversity in Brazil

Guaraci D. Cordeiro<sup>1</sup>, Patrick M. Consorte<sup>2</sup>, Bruno F. Marques<sup>2</sup>, Poliana P. Menezes<sup>2</sup>, Antonio J. C. Aguiar<sup>3</sup>, Tereza C. Giannini<sup>4</sup>, Luisa G. Carneiro<sup>2</sup>

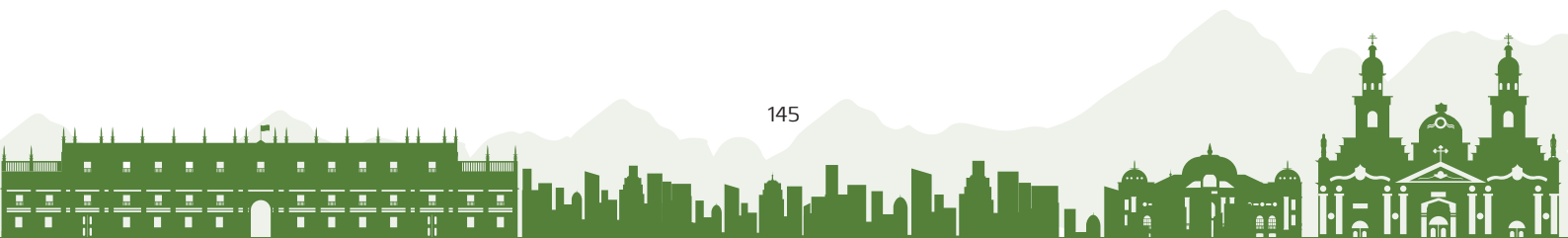
<sup>1</sup>Universidade Federal de Goiás, Goiania, Brazil, Instituto Tecnológico Vale, Belém, Brazil

<sup>2</sup>Universidade Federal de Goiás, Goiania, Brazil

<sup>3</sup>Universidade de Brasília, Brasília, Brazil

<sup>4</sup>Instituto Tecnológico Vale, Belém, Brazil

Brazil accounts for around 10% of the described world's bee species due to its continental dimension and highly diverse biomes. However, since some Brazilian biomes are still poorly sampled the current number of described bee species is underestimated. This lack of information impairs ecological studies and conservation initiatives focused on bees. Here, we used a comprehensive database of Brazilian bee occurrences (181606 valid occurrence records on 1979 species of native bees) to evaluate the extent of knowledge regarding bee diversity within Brazil. There are 2043 native species of Brazil presently described in Moure's Catalog for Neotropical bee species and recent taxonomy literature. Yet only 44% of the area of Brazil (50km cells, for 1km cells this number jumps to 99%) were sampled and we estimated that there are more than 1000 species still to be described. The Atlantic Forest has the highest number of known species (1375 species; 67% of the total number of species known in Brazil), and the lowest gap of knowledge (with 78% of the area sampled, within which only 15% of the species remain unknown). The Cerrado is the second most diverse biome in terms of known species (1091; 53%), but its knowledge gap is larger with only 50% of the area having been sampled and with 25% of the species to be discovered and described. Amazon (1071; 52%) comes in third in terms of the number of known species, but it has the largest gap of knowledge, with only 31% of the area sampled and 25% of the species still unknown. Caatinga (572; 28%) has only 53% of its area sampled, and an estimated 25% of species remain undiscovered. Pampa (478; 23%), has 56% of the sampled area and has an estimated 15% of species unknown. Pantanal (209; 10%) is one of the least-known biomes, with only 38% of the area sampled and an estimated 25% of species unknown. Overall, our results help identify biomes that should be prioritized, by further studies and investments aiming to reduce knowledge gaps on Brazilian bees (e.g. Amazon and Pantanal).





### PP-046

#### Construction of a 16S mini barcode library for french wild bees

Magalie Pichon<sup>1</sup>, Alain Vignal<sup>2</sup>, Emmanuelle Labarthe<sup>2</sup>, Nathalie Escaravage<sup>3</sup>, Geraldine Pascal<sup>1</sup>, KLOPP Christophe<sup>4</sup>, André Pornon<sup>3</sup>, Rémi Rudelle<sup>5</sup>, Anie Ouin<sup>6</sup>, Mélodie Ollivier<sup>6</sup>, Kamila Canale Tabet<sup>2</sup>

<sup>1</sup>UMR Dynafor, INRAE, 31326, Castanet-Tolosan, France

<sup>2</sup>GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France

<sup>3</sup>EDB, UMR 5174 Bâtiment 4R1 31062 cedex 9, 118 Rte de Narbonne, 31077 Toulouse, France

<sup>4</sup>UMR MIAT, INRAE, 24 Chemin de Borde Rouge, 31320 Auzeville-Tolosane, France

<sup>5</sup>Rudelide Expertise muséologie, 21 rue tour de ville, 12240 Rieuepeyroux, France

<sup>6</sup>INP-ENSAT, Av. de l'Agrobiopole, 31326 Auzeville-Tolosane, France

The use of molecular biology tools: barcoding/metabarcoding is particularly attractive and complementary to conventional methods for studying biodiversity. For wild bees, the development of DNA barcodes has been carried out in Canada and in various European countries (Germany, UK, Switzerland, etc.). In France, a national project CODABEILLES, has been initiated in 2021 to barcode the 968 species listed. Most barcoding and metabarcoding experiments on wild bees have been performed using 650 bps of the CO1 gene. Despite high amplification and sequencing success (about 70%) for most bee's families, some genera like *Andrena*, or old specimens from collections are difficult or even impossible to amplify with classical Folmer primers. In the present work, we have built a 16S (250 bp) mini DNA-barcode database. Data come from a regional collection of French wild bees that contains about 8000 individuals belonging to 174 species and 21 genera previously identified by taxonomists. The specimens had been sampled between 2013 and 2020 using pan traps or sweep nets and kept dried in the collection. DNA was extracted from front legs (one to three individuals per species) and sequenced using MiSeq and Sanger technologies. All mini-barcodes were then validated by distance tree inference. To assess the discrimination strength metabarcoding we have manually mix 3, 5, or 14 legs in variable proportion corresponding to intraspecies or interspecies bees. We demonstrated that the 16S mini barcode is well adapted to delineate wild bee species, particularly whenever CO1 is unsuccessful. Moreover, this mini barcode should be useful to perform low-cost metabarcoding and therefore opens opportunities for environmental DNA approaches by analysis of the traces left on foraged flowers.

### PP-047

#### Identification of wild and domestic bees by non-destructive molecular methods

Magalie Pichon<sup>1</sup>, Mélodie Ollivier<sup>2</sup>, Elisa Simon<sup>3</sup>, Emmanuelle Labarthe<sup>3</sup>, Alain Vignal<sup>3</sup>, KLOPP Christophe<sup>4</sup>, Anie Ouin<sup>2</sup>, Kamila Canale Tabet<sup>3</sup>

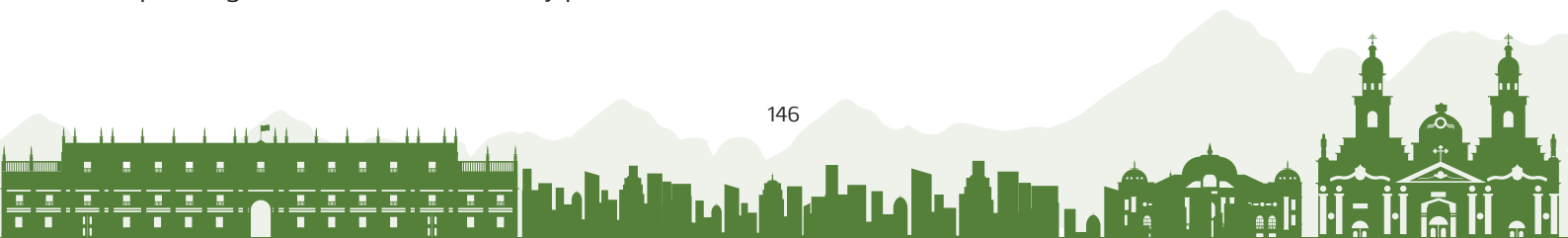
<sup>1</sup>UMR Dynafor, INRAE, 31326, Castanet-Tolosan, France

<sup>2</sup>INP-ENSAT, Av. de l'Agrobiopole, 31326 Auzeville-Tolosane, France

<sup>3</sup>GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France

<sup>4</sup>UMR MIAT, INRAE, 24 Chemin de Borde Rouge, 31320 Auzeville-Tolosane, France

The study of insect pollinator communities is at the heart of a great paradox: today, characterizing the diversity of these species implies sacrificing them (trapping and then morphological identification). Methods based on the amputation of a member (segment of antennae, piece of wings or legs) have been used in honey bees (Madella et al., 2021) or bumblebees (Holehouse et al., 2003) but they lead to mortality rates depending on the size of the specimens. Recently, different laboratories around the world are mobilizing to try to develop new non-lethal identification methods such as acoustics (Heise et al., 2020), deep learning: mathematical algorithms capable of identifying specimens from insect photos (Høye et al., 2021), and sampling from faeces (Scriven et al., 2013). At the same time, with rapid advances in sequencing methods, environmental DNA-based approaches (i.e., identification of individuals from traces left in the environment) have opened up promising prospects for inventorying aquatic or terrestrial biodiversity while preserving the integrity of specimens (Banerjee et al., 2022). We proposed to develop a non-lethal protocol to identify wild bees from the traces they can leave while foraging on flowers. It is based on the enclosure or exposure to the open air of strawberry plants and the collection of flowers for the extraction of insect DNA traces. The experimental protocol is composed of 4 strawberry plants placed in 3 conditions: a cage with insects, a cage without insects and exposed to the open air. Initially we extracted DNA from insect tracks left on flowers only in the "bees introduced into the enclosure" condition and by testing two types of extraction kits. The extracted DNAs were then amplified by PCR with the insect 16S minibarcode (Clarke et al., 2014). We had previously constructed a barcode baseline for wild bees with this minibarcode (results in publication). Several PCR conditions allowed us to select the DNA Extraction Kit that yielded sequencable amplifiates. Sequencing of 4 samples (2 duplicates) and bioinformatics processing of the data allowed us to find the sequences of the bees that were introduced but also sequences corresponding to 16S DNA of strawberry plants.





### PP-048

#### Bee genome reconstruction through the sequencing of hive products

Kamila Canale Tabet<sup>1</sup>, Elisa SIMON<sup>1</sup>, Emmanuelle Labarthe<sup>1</sup>, Emilien Rottier<sup>2</sup>, Benjamin Basso<sup>2</sup>, Fanny Mondet<sup>2</sup>, Alain Vignal<sup>1</sup>, Thibault Leroy<sup>1</sup>

<sup>1</sup>GenPhySE, Université de Toulouse, INRAE, ENVT, 31326, Castanet Tolosan, France

<sup>2</sup>INRAE, UR 406 Abeilles et Environnement, UMT PrADE, Avignon, France

As part of a global objective of describing the population structure and genetic diversity of honeybees in Europe and North Africa, we have put a considerable effort into collecting and sequencing drones (Wragg et al. 2022). From a population geneticist's perspective, the sequencing of DNA from drones indeed has many advantages, especially the one of providing direct access to haploid material (haplotypes). One drawback however is associated with the sampling, which requires visits of each hive at specific moments to collect bee drone larvae. Recently, Bovo et al. (2020) have successfully whole-genome resequenced bees by sequencing the total DNA extracted from honey, obtaining promising results with roughly a quarter of the total sequenced reads corresponding to bee DNAs. One limitation however was the low number of samples used (only 3) and the highly variable proportion of reads corresponding to bees among these samples (from ~1% to 55%). Further developments are therefore needed before considering a wide use of honey samples in order to describe the genetic makeup of the honeybee populations. Our new project aims at optimizing and deploying a strategy for obtaining bee genetic data through a sequencing approach targeting different hive products. If mastered, the direct sequencing of hive products has many advantages. It could allow easier sampling and transfer of material between countries on the one hand, but also a better ethical and environmental value of our research on the other hand, in particular by limiting the number of bees sacrificed and the carbon footprints associated with the sampling. In addition to presenting the general strategy used in the project, our main objective is to present preliminary results regarding DNA extraction and sequencing from honey samples. Further developments of the project through the DNA extraction and sequencing of additional bee products including royal jelly, propolis and beeswax will also be discussed.

Bovo et al. (2020). <https://doi.org/10.1038/s41598-020-66127-1>

Wragg et al. (2022). <https://doi.org/10.1111/1755-0998.13665>

### PP-049

#### Molecular identification of the hygienic behavior in colonies of Africanized bees (*Apis mellifera* L.)

Tânia Patrícia Schafaschek<sup>1</sup>, Gustavo Henrique Ferrero Klabunde<sup>2</sup>, Candida Elisa Manfio<sup>3</sup>, Adriana Pereira<sup>3</sup>, Adriana Santana Klock<sup>3</sup>, Sandra Denise Camargo Mendes<sup>4</sup>

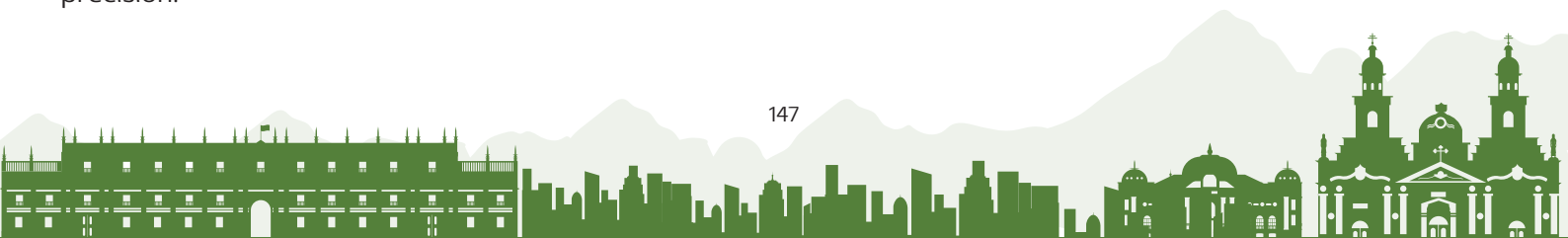
<sup>1</sup>Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), Videira Experimental Station, Videira-SC, Brazil

<sup>2</sup>Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), Department of Research and Innovation Management, Florianópolis-SC, Brazil

<sup>3</sup>Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), Itajaí Experimental Station, Itajaí-SC, Brazil

<sup>4</sup>Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), Lages Experimental Station, Lages-SC, Brazil

Hygienic behavior (HB) allows bees to resist various diseases. This characteristic can be quantified through field tests, but the use of molecular markers can improve the effectiveness of selection. In this work the molecular identification of the colonies of the Africanized *Apis mellifera* bee selection project for disease resistance and high productivity of the Epagri Experimental Station of Videira, SC, was carried out. The phenotypic evaluation of this characteristic was made by the percentage of dead offspring removed in 24 hours, using the brood freezing method. The colonies were evaluated from 2017 to 2021 in the four seasons of the year, and at each year, the colonies with the highest averages of HB had their queens reproduced to replace those with less satisfactory production characteristics and less HB. For the molecular analyses, bee samples were collected from 45 colonies that had the highest HB averages in November 2018, which were analyzed in duplicate (n=90). In the molecular evaluation of the HB of the bees, a SNP associated with the uncapping and removal behaviours that are components of the hygienic behavior was evaluated via PCR. After amplification, digestion was performed with the restriction enzyme Hpy188i and subsequent gel electrophoresis to distinguish the bands of 207 bp (uncut, hygienic) from 178 bp (cut, unhygienic). Of the 90 samples analyzed: eight were heterozygous, and 79 were homozygous for allele 207, being considered hygienic samples. No homozygous sample was observed for the unhygienic allele 178. The mean HB obtained by the brood freezing method was 82.22%; 88.39%; 92.11%; 87.74% and 91.27% for the years 2017, 2018, 2019, 2020 and 2021 respectively. The overall average of HB in the squad over the period evaluated was 89.04%. The use of the mass selection of the colonies based on the phenotypic characteristics of HB allowed the increase of the genotype in the group of colonies evaluated. The use of molecular marker combined with the use of the restriction enzyme Hpy188i was efficient for the effectiveness of the selection of bees with hygienic behavior in the colonies studied, assisting in decision making with more speed and precision.



### PP-050

#### Gibberellic acid alters morphology and expression of the vitellogenin gene (VG) in *Apis mellifera* honeybees

Gustavo Henrique Simões Pereira<sup>1</sup>, Adriana A. Sinópolis Giglioli<sup>2</sup>, Daiani Rodrigues Moreira<sup>2</sup>, Cinthia Leão Figueira<sup>1</sup>, Angélica de Souza Khatlab<sup>1</sup>, José Washington Santos Oliveira<sup>1</sup>, Tuan Henrique Smielewski de Souza<sup>1</sup>, Douglas Galhardo<sup>1</sup>, Sandra Milena Diaz Puentes<sup>1</sup>, Eliane Gasparino<sup>1</sup>, Maria Claudia Colla Ruvolo-Takasusuki<sup>2</sup>, Vagner de Alencar Arnaut de Toledo<sup>1</sup>

<sup>1</sup>Department of Animal Science, State University of Maringá, 87020-900, Maringá, Paraná, Brazil

<sup>2</sup>Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, 87020-900, Maringá, Paraná, Brazil

*Apis mellifera* collects nectar and pollen, important sources of carbohydrates, proteins, lipids, vitamins, minerals, essential to promote bee development and productive performance of the colonies. Furthermore, terpenoids, secondary metabolites and phytochemicals produced by plants can also be found in floral resources. Among the phytochemicals, gibberellic acid (GA3), a plant growth hormone, has been investigated, due to its chemical similarity to the juvenile hormone (JH). However, the metabolic process of GA3 in pollinating insects is still unclear. Thus, this study aimed to investigate the effects caused by the ingestion of different concentrations of GA3 by *Apis mellifera* in morphological changes in midgut and fat body and expression of the Vitellogenin (VG). For that, newly emerged workers were divided into 25 cages with an average population of 150 worker honeybees. The cages were divided into five treatments: No addition of gibberellic acid in the feed (CTRL), 0.45 mg/g of GA3 in the feed (DT045), 0.90 mg/g of GA3 in the feed (DT09), 1.35 mg/g of GA3 to the feed (DT135) and 1.80 mg/g of GA3 to the feed (DT180). The bees were kept at a temperature of  $34 \pm 2^\circ\text{C}$ , with a relative humidity of  $60 \pm 10\%$  during seven days. At the end of the experimental period, the bees were euthanized by freezing and CO<sub>2</sub> and then dissected for tissue extraction used in histology and gene expression analysis. Histological analyzes showed the effect of gibberellic acid on the intestinal epithelium and trophocytes of the fat body, indicating the inability to absorb nutrients from the diet of insects subjected to low GA3, while the analysis of oenocytes and pericardial cells denote the detoxification process triggered by high concentrations of GA3 in the diet. The reduction in the expression levels of the VG gene may be associated with its degradation occurring by esterases that have the juvenile hormone (JH) as a target, thus causing hormonal disturbances that could reflect a reduction in longevity, however further investigations is necessary.

### PP-051

#### Does the stingless bee *Melipona bicolor schencki* choose the tree species to build its nest in southern Brazil?

Sidia Witter<sup>1</sup>, Leticia Azambuja Lopes<sup>2</sup>, Claudio Augusto Mondin<sup>3</sup>, Bruno Lisboa<sup>1</sup>, Betina Blochtein<sup>4</sup>, Vera Lucia Imperatriz Fonseca<sup>5</sup>

<sup>1</sup>Secretaria de Agricultura, Pecuária, Desenvolvimento Sustentável e Irrigação do Rio Grande do Sul Brasil

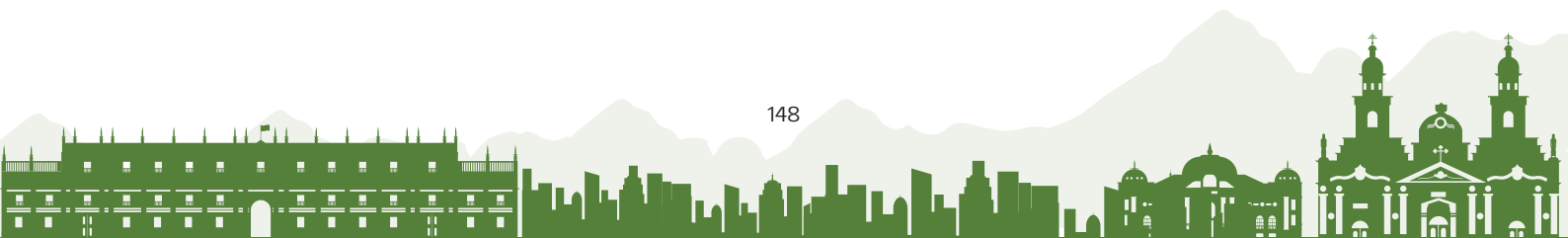
<sup>2</sup>Universidade Luterana do Brasil

<sup>3</sup>Instituto Conectar Ambiental

<sup>4</sup>Mais Abelhas Consultoria Ambiental

<sup>5</sup>Instituto de Biociências da Universidade de São Paulo

The conservation of Meliponini and their ecosystem services require studies on potential nesting sites that can be addressed to the implementation of friendly practices and public policies to prevent the decline of their populations and sustainable use. Nests of *Melipona bicolor schencki* (Meliponini) were studied in the Araucaria Forest in Cambará do Sul/Rio Grande do Sul/Brazil to verify the preferred tree species for nesting and their relationship with the abundance of tree species in the area. For the qualitative and quantitative study, the plot method was adopted, sampling all tree individuals with a diameter at breast height (DBH)  $\geq 25$  cm present in 38 plots of 100 m<sup>2</sup> each, in two forested areas according to the presence (area 1) or absence of nests (area 2). Trees containing nests, distributed over 250 ha, were identified. In area 1 with rugged, sloping relief, drained soil where cattle traffic was very rare, 20 botanical species from 12 families with an average DBH of 35.9 cm were recorded. The most abundant botanical family in this area was Lauraceae (28%) and the species *Ocotea* sp. (20%). Area 2, on the other hand, with flat relief, very humid soil and with a cattle use, was found nine botanical species from eight families, with an average DBH of 32.2 cm. Araucariaceae (*Araucaria angustifolia*) and Aquifoliaceae (*Ilex microdonta*) represented 41.4% of the trees species in the area. 55 nests of *Melipona bicolor schencki* were recorded in trunks of 16 tree species from 11 families: 63% of these in Lauraceae and *Cinnamomum amoenum* with the highest percentage of nests (40%). The average DBH of tree species containing nests was 46 cm, and 70% of the nests were found in trees with DBH  $\geq 40$  cm. The results would justify the preference of *Melipona bicolor schencki* for nesting in Lauraceae, the most abundant family in area 1, but would not explain its preference for *Cinnamomum amoenum*, since this species corresponds to only 2% of the relative density of the tree component.



### PP-052

## Agricultural intensification impairs cognitive abilities and the expression of genes associated with social responsiveness in honeybees

Ivana N Macri<sup>1</sup>, Pablo J Moja<sup>2</sup>, Ignacio Rivas Fontan<sup>4</sup>, Jose M Latorre Estivalis<sup>4</sup>, Diego Cristos<sup>3</sup>, Jorge A Zavala<sup>5</sup>, Walter M Farina<sup>4</sup>

<sup>1</sup>Laboratorio de Insectos Sociales, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina. Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina. Instituto de Ingeniería Rural, Centro de Investigación de Agroindustria (CIA), Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Buenos Aires, Argentina.

<sup>2</sup>Laboratorio de Insectos Sociales, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina. Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina, EEA Cuenca del Salado. Instituto Nacional de Tecnología Agropecuaria (INTA). Rauch, Buenos Aires, Argentina.

<sup>3</sup>Instituto Tecnología de los Alimentos, Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Buenos Aires, Argentina.

<sup>4</sup>Laboratorio de Insectos Sociales, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina. Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina.

<sup>5</sup>Cátedra de Bioquímica, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina, Instituto de Investigaciones en Biociencias Agrícolas y Ambientales, (INBA), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina.

The honeybee *Apis mellifera* is one of the main pollinators in agroecosystems, and as such, its colonies are exposed to agricultural intensification. This leads to increased use of agrochemicals and a reduction in floral diversity, resulting in fragmented and more homogeneous habitats. The resources they obtain in these environments may be exposed to agrochemicals that can accumulate in their colonies and be distributed among their members. In the present study, a behavioral, sociobiological and molecular approaches were used to compare the global state of beehives at two different times of crop management in a field of the province of Buenos Aires, Argentina. These issues were correlated with the availability of resources, the heterogeneity of the environment and the application of agrochemicals, and they were done for two summer seasons. From a behavioral approach, on the one hand, we studied the foraging activity of the colonies (incoming bees with collected pollen loads). On the other hand, using the proboscis extension response (PER) in forager bees captured in the different apiaries, sucrose responsiveness and olfactory learning abilities were evaluated. From a molecular approach, the presence of pesticide residues in the pollen collected was analyzed. Furthermore, the relative expression of genes associated with the genetic basis of sociability in bees was evaluated in foragers. Our results confirm that traces of agrochemicals are entering into the colonies and suggest an intricate relationship between pesticide traces in food resources, cognitive abilities, nutritional status and relative gene expression in the honeybee brains. Exposure to agricultural intensification by the presence of pesticide residues in pollen and by the decrease in the quantity, variability and quality of nutritional resources for colonies, impairs the cognitive abilities of forager bees. In addition, agricultural intensification leads to a decrease in the expression profiles of genes related to different metabolic and neuronal pathways that are associated with the genetic basis of bee sociability.

### PP-053

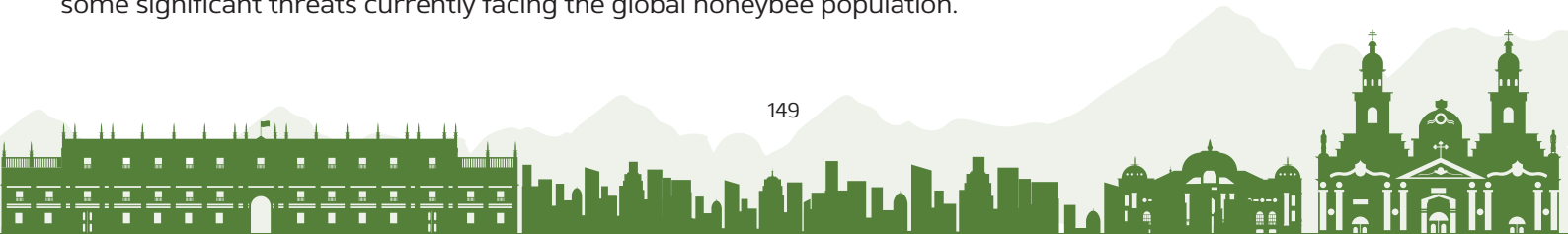
## Deep Learning for Queen Bee Detection in Honeybee Hives using biological signals

Pablo Ormeno-Arriagada

Universidad Técnica Federico Santa María, Valparaíso, Chile

Honeybees play a crucial role in maintaining the ecosystem balance and supporting human activities such as agriculture. However, the global honeybees population has been declining in recent years, meaning a significant threat to biodiversity and economic sustainability. In this context, improving beekeeping practices through the use of advanced technologies has become a critical research priority. One important area of investigation is anomaly detection within beehives, which can help beekeepers to identify and address issues such as the loss of a queen bee or the presence of parasites.

This study aimed to contribute to the development of more effective anomaly detection techniques for beekeeping by applying deep learning methods to the problem of queen bee detection. Specifically, a deep learning convolutional neural network was trained using a signal characterized by the Mell logarithmic spectrogram and cepstral coefficients at Mel frequency extracted from the amplitude time series and Mel spectrogram. These features were selected based on their ability to capture relevant information about the presence or absence of a queen bee. The resulting model achieved good results in terms of accuracy and efficiency. In particular, it is faster, consumes less memory, and achieves better classification performance metrics compared with other approaches. These findings suggest that deep learning approaches can be valuable tools for improving bee-keeping practices and contributing to the preservation of honeybee populations. The benefits of the proposed model are not limited to queen detection alone. The methodology employed in this study could be adapted to detect a range of other anomalies, such as the presence of *Varroa*, which is a significant threat to honey health. By enabling beekeepers to identify and address such issues more quickly and effectively, deep learning approaches have the potential to significantly improve the sustainability and productivity of beekeeping operations. In conclusion, this study emphasizes the potential of deep learning approaches for improving beekeeping practices and contributing to the preservation of the honeybee population. By providing more efficient and accurate means to detect anomalies within beehives, these methods could mitigate some significant threats currently facing the global honeybee population.



### PP-054

#### Innovations in honey bee ethology during propolis harvesting

Roman Dvykaliuk<sup>1</sup>, Leonora Adamchuk<sup>2</sup>

<sup>1</sup>National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

<sup>2</sup>National University of Life and Environmental Sciences of Ukraine, National Science Center "PI Prokopovich Institute of Beekeeping", Public Organization «Foundation of Women Beekeepers», Kyiv, Ukraine

Propolis is a sticky resinous substance collected by bees from the buds, leaves, and stems of wild plants. Bees process it and use it to seal cracks in their hives, polish wax cells, and embalm the corpses of their enemies. Propolis has antibacterial properties and a density of  $0.953 \pm 0.001 \text{ g/cm}^3$ . At  $24^\circ\text{C}$ , the average Young's modulus of propolis is  $11.23 \pm 6.77 \text{ MPa}$  ( $N=45$ ). However, a  $2^\circ\text{C}$  temperature increase leads to a significant decrease in elasticity to  $6.55 \pm 4.89 \text{ MPa}$  ( $N = 10$ ,  $P = 0.044$ ). Propolis has an average adhesion work of  $2.96 \pm 1.27 \text{ J/m}^2$  and an average breakout force of  $2.12 \pm 0.77 \text{ mN}$ . Its adhesion is reduced by four times in the presence of natural secretion on the epicuticle of the lower jaw, and a scaly micropattern on the jaw can contribute to the spread of fluid. A low contact angle ( $< 30^\circ$ ) of the substance suggests that the surface of the mandible may be oleophilic (Saccardi et al. 2022). Our study aimed to examine the devices developed to obtain propolis and bees' behavior during its deposition. Honey bees dilute propolis, forming drops on a wooden surface with a diameter of  $35.7 \mu\text{m}$  to  $1500.0 \mu\text{m}$  ( $N=450$ ). The average drop diameter in the experiment was  $562 \mu\text{m}$  ( $N=450$ ), and the deviation of the contact line of the drop from roundness was  $39.0 \mu\text{m}$  ( $N=450$ ), and the distance from the edge of the glass substrate to the center of the drop averaged  $27944.0 \mu\text{m}$  ( $N=450$ ). **CONCLUSION:** Bees use their entire mouthparts when manipulating diluted propolis. The formations that they create in the openings of the nets have an arcuate shape. The inner part of the arch corresponds to the side of the net that bees have access to, and the outer part of the arch is formed from the side to which they do not have access. These arches have natural strength, which bees use to create stable structures. So, we have another shape besides the hexagon when building honeycombs.

### PP-055

#### Understanding fermentation processes in stingless bees: implications for developing fermented feeds

Patrícia Miranda Pinto<sup>1</sup>, Leandro Barros De Souza<sup>2</sup>, Luciana Teresa Dias Cappellini<sup>3</sup>, Michelle Manfrini Morais<sup>4</sup>

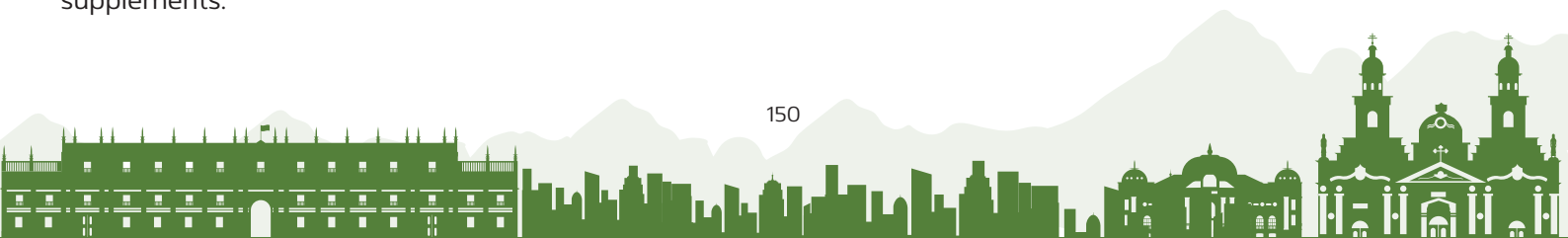
<sup>1</sup>Programa de Pós Graduação em Biotecnologia, Universidade Federal de São Paulo, São Paulo, Brazil.

<sup>2</sup>Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, São Paulo, Brazil.

<sup>3</sup>Department of Chemistry & Biochemistry, Florida International University, Florida, United States of America

<sup>4</sup>Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Paulo, São Paulo, Brazil.

Stingless bees (Apidae, Meliponini) constitute an important group of eusocial insects found in tropical and subtropical regions of the world. To endure intervals of paucity, these bees store nectar and pollen from flowers. Both nectar and pollen undergo fermentation processes during storage, resulting in honey and fermented pollen, respectively. However, little is known about the fermentation process with the pollen collected by stingless bees during storage. Understanding these processes not only clarifies questions about how pollen fermentation occurs in different species of stingless bees but also helps to shed light on important issues related to the transformation process of pollen, which can be applied in the production of fermented feeds using specific microorganisms. Therefore, the objective of this study was to observe pollen storage behavior in the pots within the colonies to obtain information that could help in understanding the fermentation process and subsequently be used in applied research for the development of fermented feeds for bees. The observation was conducted for 30 days, using a *Melipona quadrifasciata* colony from the Experimental Meliponary of the Universidade Federal de São Paulo, on the Diadema campus (Brazil – SP). Observations were carried out every day, in the morning and evening. The pollen pots observed in this study were marked with a random combination of colors, to differentiate them more easily and collect data regarding the work dynamics performed by worker bees. The observations revealed no pattern regarding the opening and closing dynamics of the pots and that the worker bees constantly moved in and out of the open pollen pots. This event may indicate a continuation of the fermentation process regarding gas exchange and maintenance of the flow of nutrients. Based on this study, it was concluded that in the case of the storage and treatment of pollen in *M. quadrifasciata* species of bees, the lack of regularity in handling this food resource is remarkable. Data such as this is crucial for advancing techniques that enhance meliponiculture globally and locally, including creating fermented protein supplements.





### PP-056

#### DNA content and genotyping of the honeybee spermatheca

Andraž Marinč<sup>1</sup>, Anja Pavlin<sup>2</sup>, Ajda Moškrič<sup>1</sup>, Katarina Mole<sup>1</sup>, Jernej Bubnič<sup>1</sup>, Andreja Opara<sup>1</sup>, Marin Kovačič<sup>3</sup>, Zlatko Puškadija<sup>3</sup>, Aleksandar Uzunov<sup>4</sup>, Sreten Andonov<sup>5</sup>, Bjørn Dahle<sup>6</sup>, Janez Prešern<sup>1</sup>

<sup>1</sup>Agricultural institute of Slovenia, Slovenia

<sup>2</sup>Biotechnical Faculty, University of Ljubljana, Slovenia

<sup>3</sup>Centre for Applied Life Sciences Healthy Food Chain, Croatia

<sup>4</sup>Company for Applied Research and Permanent Education in Agriculture, Skopje, Macedonia

<sup>5</sup>Swedish University of Agricultural Sciences, Sweden

<sup>6</sup>Norwegian Beekeepers Association, Norway

In our study, we sought to optimize short-term storage methods for preserving, transporting, and retrieving genetic information from honeybee queen spermatheca. By genotyping the contents of the spermatheca, we can evaluate mating control success and genomic selection more efficiently than through individual offspring genotyping. To achieve this goal, we examined the suitability of different short-term storage conditions and DNA extraction techniques for subsequent DNA sequencing and microsatellite analysis.

We tested six different storage conditions for the spermatheca, using either Allprotect Tissue Reagent or absolute ethanol as a preservation agent, and simulated transport conditions by keeping samples at room temperature for 6-8 days. We analyzed the concentration of DNA, RNA, and proteins in the DNA extracts, and assessed the degree of DNA fragmentation through the amplification of genetic markers. We also tested the suitability of the extracted DNA for microsatellite analysis.

Our results showed that storing spermatheca in absolute ethanol was the most suitable for preserving DNA, due to its availability, low cost, and simplicity of use. Although there were differences in DNA yield from the samples subjected to different storing conditions, there was no significant effect of storage method or DNA extraction technique on the amplification success. However, fewer samples stored in ethanol amplified successfully compared to those stored in Allprotect Tissue Reagent.

Finally, we tested the use of spermatheca as a source of patriline composition in an experiment with instrumentally inseminated virgin queens, and performed microsatellite analysis of the extracted DNA from each spermatheca, as well as queens' and drones' tissue.

Overall, our research provides guidance for future genotyping studies of entire honeybee colonies, which could have significant implications for advancements in genomic selection and mating control evaluation.

### PP-057

#### Aspects of the threats to the Carniolan bee ("*Apis mellifera carnica*") in Slovenia

Dr Petra Bole

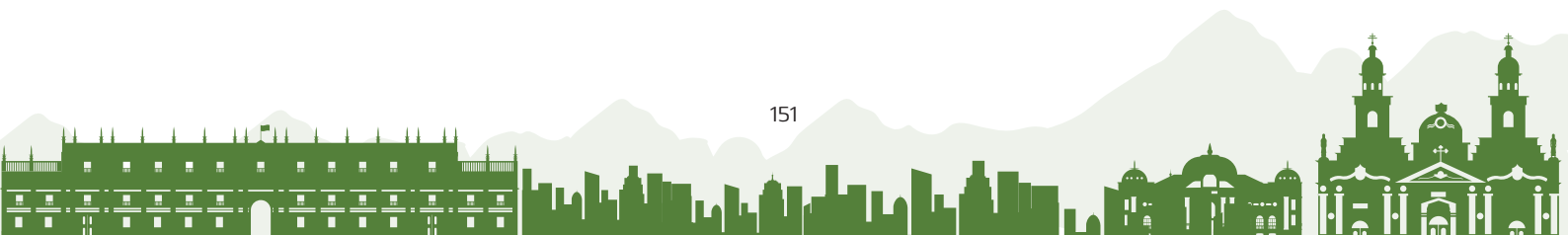
Museum of Apiculture, Radovljica, Slovenia

The accelerated rate at which bees are dying has long ceased to be shocking, as it is being constantly reported from various parts of the world. There is a lot of discussion about the threats to the Carniolan bee (*Apis mellifera carnica*) in Slovenia and the search for reasons for the situation. In this article, we analyse some of the negative impacts that affect the Carniolan bee.

An analysis of the aspects of the threats to the Carniolan bee at the time of its accelerated decline shows that these threats are, among other things, multi-faceted genetic mixing and the decline in local bee populations as a result of the reduction of their genetic diversity. The gene pool is becoming depleted, therefore it is necessary to preserve the Carniolan bee at the level of local variations or ecotypes. Due to natural barriers, individual populations became isolated, the gene flow between them was no more, and they developed into independent subspecies or geographic races.

The Carniolan bee is protected by law in Slovenia, and it is the only bee that can be used for beekeeping. Despite the legal ban, there have been cases of foreign queens being imported, which may have been sold as Carniolan bees, although their origin is unclear. Some beekeepers import queens of the Italian bees, also Buckfast bees. The introduction of the Buckfast bee poses a threat of genetic pollution as such, and unfavourable ethological changes may occur in the offspring of the aforementioned hybrids and the Carniolan bee.

There are around 30 registered breeders of queens in Slovenia, scattered across the areas of various ecotypes of bees. Queens can be freely sold in our country, which means that beekeepers can buy queens from any breeder. Thus, bees are increasingly being interbred, their identity is becoming more and more blurred, the ability to survive in a given environment can decrease, and the gene pool is becoming ever poorer.





### PP-058

#### Climate change: tolerance, survival and dehydration of *Apis mellifera iberiensis* subspecies under different temperature and relative humidity environments

Raquel Martín Hernández<sup>1</sup>, Antonio Nanetti<sup>2</sup>, Soledad Sagastume<sup>1</sup>, Giovanni Cilia<sup>2</sup>, Amelia V. González-Porto<sup>1</sup>, Mariano Higes<sup>1</sup>, Medibees Consortium<sup>3</sup>

<sup>1</sup>CIAPA-IRIAF Centro de Investigación Apícola de Marchamalo, Marchamalo, Spain

<sup>2</sup>CREA-AA Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy

<sup>3</sup>Various institutions

Climate change is a source of new stressors for bees due to rising temperatures and changing rainfall patterns. This is not only a climatic stress per se, but also a nutritional stress as these changes affect the flowering times of many plant species, making spring earlier and summer longer. The MEDIBEES PRIMA project “Monitoring the Mediterranean honey bee subspecies and their resilience to climate change for the improvement of sustainable agro-ecosystems”, addresses the study of the effect of climate change on different subspecies of *Apis mellifera* in the Mediterranean area. One of the key points to infer the adaptability of bees to these environmental changes is the study of temperature tolerance as well as the survival capacity of the different subspecies under different temperature and humidity conditions. In this study we show the results of Spanish bees (*A. m. iberiensis*) on their tolerance to cold and heat, their survival rate under different temperature and humidity conditions, and their tendency to dehydration. The results obtained show how the honey bee of the Iberian Peninsula is less tolerant to high temperatures than other subspecies native to warmer climates, as well as the influence of age and sex on this tolerance. Likewise, the effect of temperature and humidity as independent stressors has been evaluated, as well as their synergy, since both factors together behave as an independent stressor, through survival and body water loss studies. Our results show that for *A. m. iberiensis* temperatures above 40°C are critical for survival, while high percentages of relative humidity (75%) dampen both survival and slow dehydration.

This project is part of the PRIMA programme supported by the European Union.

### PP-059

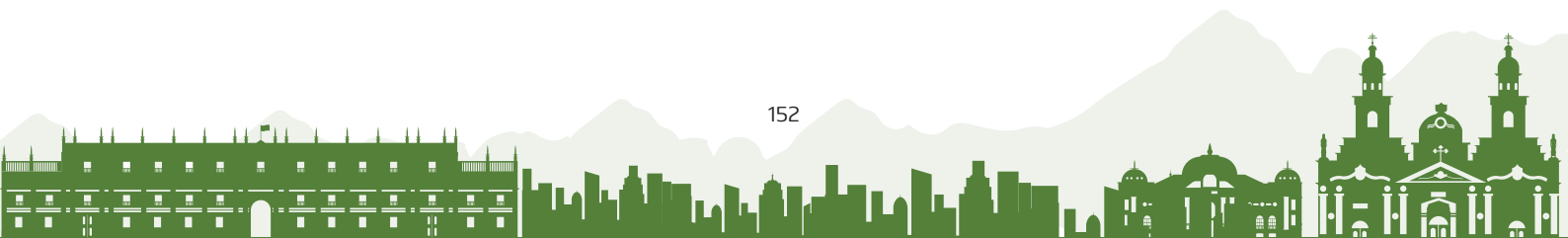
#### The Canadian Bee Gut Project

Brendan A Daisley<sup>1</sup>, Elizabeth Mallory<sup>1</sup>, Anna M Chernyshova<sup>2</sup>, Andrew P Pitek<sup>2</sup>, María Agustina Rodríguez<sup>2</sup>, Sophie M Killam<sup>2</sup>, Julia F Lacika<sup>2</sup>, Emma Allen-Vercoe<sup>1</sup>, Graham J Thompson<sup>2</sup>

<sup>1</sup>Department of Molecular and Cellular Biology, University of Guelph, Canada

<sup>2</sup>Department of Biology, Western University, Canada

The main goal of the Canadian Bee Gut Project is to define what constitutes a ‘healthy’ microbiome across starkly changing seasons and landscapes that define the Canadian beekeeping scene. This citizen science-based initiative was launched in 2022 and has so far developed a streamlined system for the strategic retrieval of bees, their guts, and associated hive data from diverse commercial Canadian operations. Our system uses return-by-mail sample tubes and hive mounted QR codes that upon scanning prompt the beekeeper to enter on-site hive and management details directly from their phone. We collect, anonymize and compile the samples and meta-data to ultimately correlate differences in honey bee gut microbiomes to colony-level outcomes year after year and region by region. Our nationwide data mapping catalogue for the honey bee metagenome is therefore vast and will enable a unified investigation of the underlying microbial interactions affecting sustainability of the Canadian beekeeping industry. Moreover, our metagenomic shotgun-sequencing and NMR-based metabolomics analysis of collected gut samples will permit the most comprehensive assessment yet of the Canadian bee microbiome taxonomic structure and its functional capacities. We expect to identify disease-causing microbes associated with colony loss but likewise can identify depletion of health-promoting symbiotic microbes or other imbalances ('missing microbes') that increase susceptibility to stressors such as pathogens and agrochemical exposure. We further expect to find previously uncharacterized honeybee-associated microbes, which could be used for new microbial therapeutic strategies in honey bees. Ultimately, the knowledge gained from this work will improve our understanding of how microorganisms impact honey bee health as well as help to guide policy geared towards sustainable management of honey bees in the agricultural sector.



### PP-060

#### Development of bumblebee colonies of *Bombus bellicosus* and *Bombus pauloensis* under two nutritional diets

Juan Ignacio Davoine, Yulai Ramos, Ciro Invernizzi, Sheena Salvarrey

Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Bumblebees of the genus *Bombus* are excellent pollinators that contribute to the maintenance of natural ecosystems and agricultural production. Uruguay has two native species, *Bombus bellicosus* and *Bombus pauloensis*, which, along with other pollinators, have their populations threatened by various causes, including lack of food diversity due to monoculture. This study evaluated the impact of nutrition on the development of bumblebee colonies. To do this, colonies were started from 52 *B. bellicosus* queens and 60 *B. pauloensis* queens using pollen from *Eucalyptus grandis* (monofloral pollen, M) and pollen from various botanical sources (polyfloral pollen, P) as a substrate for queens to start the nest and then to feed the larvae. Colony development (oviposition, worker birth, time from oviposition to birth, worker weight) was determined until the colony had at least 10 workers. Of the total queens collected in each group, between 48% and 61.5% initiated oviposition, with no differences found between species or treatments. The time elapsed from oviposition to the birth of the first worker was shorter in *B. pauloensis* with monofloral pollen than with polyfloral pollen ( $24.86 \pm 2.44$  and  $28.29 \pm 6.73$  days, respectively) ( $P=0.01$ ). In *B. bellicosus*, no differences were found ( $28.29 \pm 1.48$  monofloral and  $29.23 \pm 1.53$  polyfloral) ( $P=0.97$ ). The average weight of *B. pauloensis* workers with monofloral pollen was higher than with polyfloral pollen ( $0.15 \pm 0.05$  and  $0.12 \pm 0.04$  g, respectively) ( $P=0.01$ ). In *B. bellicosus*, no differences were found ( $0.13 \pm 0.02$  and  $0.13 \pm 0.01$ ) ( $P=0.32$ ). The results found, although unexpected since bumblebees usually exploit different floral resources simultaneously, show that the botanical origin of pollen can affect colony development and worker size in some bumblebee species.

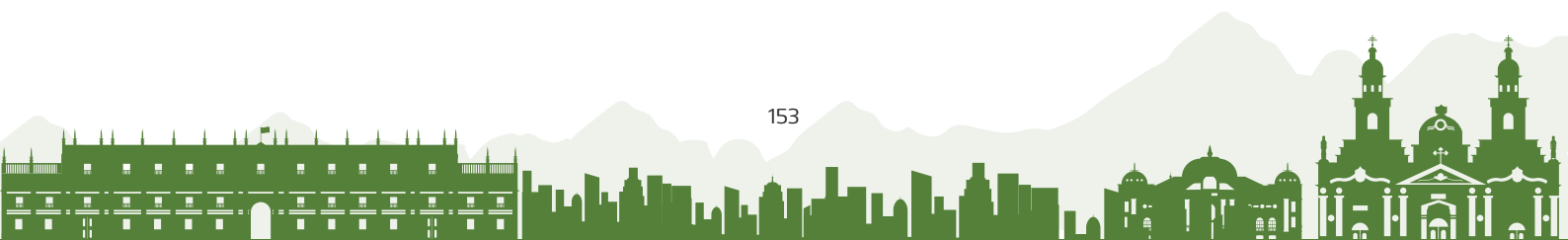
### PP-061

#### Effect of packaging on the texture and dry matter of the protein supplement prosisa® in commercial nucleus colonies of *Apis mellifera*

Yhann Vallenás-Sánchez, Nikol Siche

CAPDES, Department of agronomy and zootechnics, National university of Trujillo, Trujillo, Perú.

Both climate change and loss of natural habitat negatively impact pollen quantity and diversity. This reduces the position of the queen, the population of the colonies, and the productive parameters of the hives. Faced with this reality, protein supplements are used to reduce the negative impact of pollen scarcity. However, protein supplements lose moisture which can affect their consumption. To the authors' knowledge, this is the first study to evaluate the effect of packaging on the texture of supplements. The present study was carried out during the months of March-April (autumn-winter) of the year 2023. 10 commercial nucleus colonies of *Apis mellifera* installed in a polyfloral landscape were chosen. These colonies received two treatments (WOP: patty without packaging and WP: patty with packaging) and both treatments contained sugar syrup as a stimulant (2kg sugar/1kg of water). At the end of the two weeks, samples of ~40g were taken, with a diameter of 42mm and a thickness of 7mm. These samples were analyzed by texturometer using the following parameters: Test speed=1mm/sec, compression distance=4mm, load cell=5kg/f, and test piece= P/2. Our results showed that there was a statistical difference in dry matter (WOP:90.913 and WP:90.239, p-value=0.047) and hardness (WOP:187.775 and WP:111.504, p-value=0.0044). However, cohesiveness (WOP:0.371 and WP:0.547, p-value=0.0509), adhesiveness (WOP:147.231 and WP:141.216, p-value=0.8636), elasticity (WOP:0.845 and WP:0.911, p-value=0.3452), gumminess (WOP:67.762 and WP:58.464, p-value=0.4447) and chewiness (WOP:59.447 and WP:53.010, p-value=0.6478) were similar in both treatments. In conclusion, the use of packaging decreased the dry matter and hardness of the protein supplement. We recommend comparing the effect of packaging on the texture and dry matter of supplements in different seasons.



### PP-062

## Towards a more sustainable future: our multidisciplinary approach based on an innovative hatchery prototype for queen bee production

Mario Sarango Mogollón, David Llacsahuache Cabrera, Victor Miguel Seminario Rivera

Research and Development Area, Company D'Calidad Peru, Piura, Perú

Bees are incredible beings with a millenary evolution that give us the gift of their presence in the world to work with them. Our main priority is to work in collaboration with nature and to achieve sustainable and responsible management. To achieve this, we have developed a multidisciplinary project that aims to improve breeding and guarantee the production of queens that will ensure the future of the new colonies.

Our approach focuses on simulating natural conditions in the hatchery, providing a controlled environment conducive to cell development and successful queen hatching. We have designed a prototype incubator that uses digital and interactive devices to simulate, sense and control real-world objects in the hive, using electronics and embedded systems programming.

The electronic part of the incubator plays a fundamental role in optimising the incubation process by obtaining the precise magnitudes of temperature and humidity through its integrated sensors, and acting on the respective actuators immediately and accurately. In addition, its user-friendly interface allows the beekeeper to easily adjust the temperature and humidity parameters according to their specific needs through an app that communicates with the incubator via Bluetooth technology. The incubator also features a high-quality LCD display, which provides real-time information on the current temperature and humidity in the chamber, allowing for continuous monitoring and storage of accurate information on incubation conditions.

The incubator design has been developed through a 3D modelling process, which has allowed for optimal integration of each part and device into a highly versatile architecture. This modularity gives beekeepers the flexibility to adapt the incubator to different production scenarios, according to their specific needs.

In addition, we are working on research and development (R&D) to constantly improve the processes and technologies used in the incubator, including the implementation of a climate-controlled system and other specific projects aimed at maximising the efficiency and quality of queen production. We are committed to continue working on technologies and processes that contribute to the welfare of bees and the success of sustainable beekeeping, as this is essential to ensure the future of bee colonies and the environment in general.

### PP-063

## Addressing knowledge gaps on stingless bees: A global data review

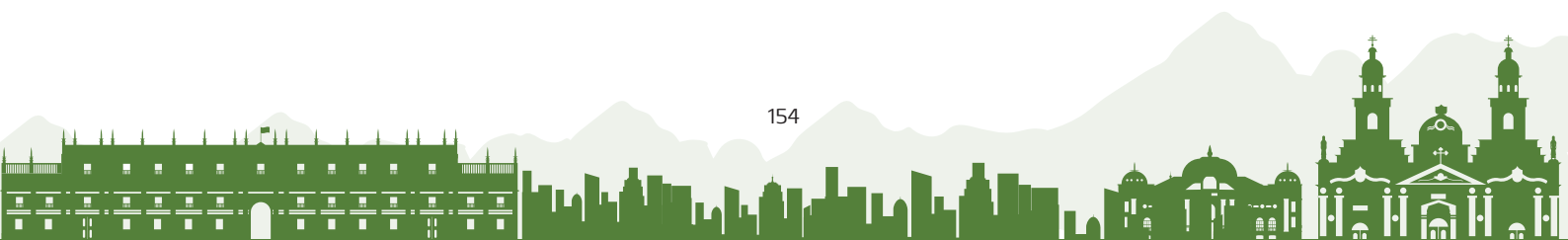
Denise Araujo Alves<sup>1</sup>, Tereza Cristina Giannini<sup>2</sup>, Marcelo Awade<sup>2</sup>, José Mauricio Simões Bento<sup>1</sup>, Vera Lucia Imperatriz-Fonseca<sup>3</sup>

<sup>1</sup>Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo, Av. Pádua Dias, 11, Piracicaba, 13418 900, Brazil

<sup>2</sup>Vale Institute of Technology - Sustainable Development (ITV-DS), Rua Boaventura da Silva 955, Belém, 66055 090, Brazil

<sup>3</sup>Department of Ecology, Bioscience Institute, University of São Paulo, R. do Matão Trav. 14 321, São Paulo, 05508 900, Brazil

Stingless bees are an important resource to meet the growing agricultural demand for pollination, as they ensure efficient and effective sexual reproduction of many wild plants and commercial crops in tropical region. Due to their remarkable features, stingless bees are an excellent option for crop pollination and honey production (and other bee products), mainly for smallholders, making them attractive both to public and private initiatives. The growth of the stingless beekeeping industry (meliponiculture) requires the optimization of management practices to increase the production of bee products and, consequently, the income source of beekeepers. However, there are substantial gaps in our knowledge regarding basic biology and technical skills. In this context, we carried out an extensive literature search on global meliponiculture and biological aspects of stingless bees to identify these knowledge gaps, considering the different regional characteristics related to cultural, economic and ecological factors. This is the first large-scale effort to optimize meliponiculture using a quantitative approach. From the 749 studies for which we compiled information, 242 are related to aspects of nesting biology and management, 453 studies with data related to bee-plant interactions and 100 studies with information on tree species that provide cavities that shelter stingless bee nests. This survey highlights current knowledge gaps about biology of the vast majority of stingless bee species, as well as their potential use, in all tropical regions where they occur.



### PP-064

#### Expression analysis of candidate genes associated with grooming behavior in *A. mellifera*

Romina Russo<sup>1</sup>, Maria Clara Liendo<sup>1</sup>, Irina Muntaabski<sup>1</sup>, Lucas Landi<sup>2</sup>, Hernán Pablo Pietronave<sup>3</sup>, Julieta Merke<sup>4</sup>, María Alejandra Palacio<sup>5</sup>, Graciela Adriana Rodriguez<sup>6</sup>, Alicia Mabel Basilio<sup>7</sup>, Silvia Lanzavecchia<sup>1</sup>, Alejandra Carla Scannapieco<sup>1</sup>

<sup>1</sup>IGEAF, "E. A. Favret"-(INTA) – Grupo Vinculado al IABIMO(CONICET). Hurlingham, Buenos Aires, Argentina.

<sup>2</sup>IRB, INTA. Hurlingham, Buenos Aires, Argentina.

<sup>3</sup>EEA. Reconquista, INTA. Santa Fe, Argentina.

<sup>4</sup>IdiCal (INTA-CONICET). Santa Fe, Argentina.

<sup>5</sup>EEA. Balcarce, INTA, Buenos Aires, Argentina

<sup>6</sup>EEA. Ascasubi, INTA, Buenos Aires, Argentina.

<sup>7</sup>Cátedra de Avicultura, Cunicultura y Apicultura, FAUBA, UBA. Buenos Aires, Argentina

*Varroa destructor* is one of the greatest threats to the health of *Apis mellifera* colonies. This ectoparasitic mite has negatively impacted honey production and crop pollination, generating large economic losses. Grooming behavior involves the ability of worker bees to detect and remove the mite from their own or their colony mate's bodies. This behavior can confer resistance to *V. destructor* and shows phenotypic and genetic variation among honey bee colonies. Previous studies have proposed candidate genes associated with grooming behavior. Our objective was to evaluate candidate genes and analyze their expression profiles in *Varroa* artificially infested bees from selected colonies that exhibited intense and light grooming behavior. Five candidate genes (*Nrx1*, *CYP9Q3*, *Oa1*, *pUf68*, and *at11*) were analyzed by qPCR. Differential levels of expression were detected between the compared bee groups. Specifically, intense-grooming bees exhibit a higher expression of *Nrx1*, *CYP9Q3*, *Oa1*, and *pUf68* genes and lower levels of *at11* gene in response to the mite compared to light-grooming bees. These results suggest that these genes are potentially associated with the molecular mechanisms modulating the expression of this grooming phenotype supporting novel approaches to the conservation and selection of honey bee stocks for beekeeping.

### PP-065

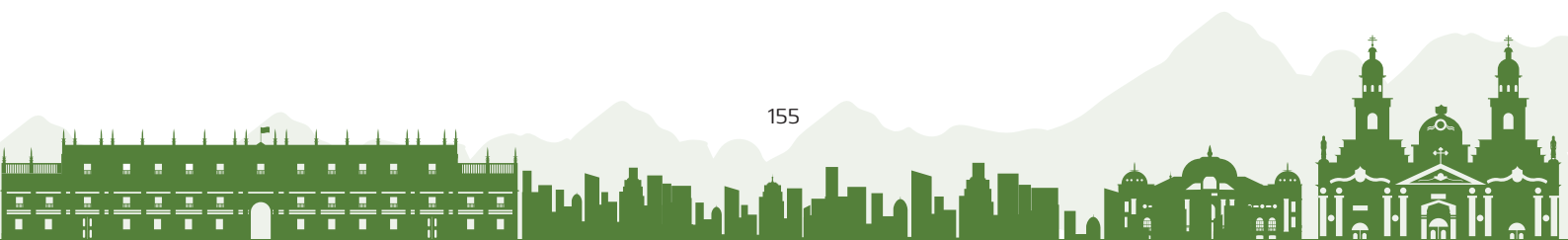
#### Thermoregulatory mechanisms in africanized bees managed under high temperatures and intense solar radiation

Katia Peres Gramacho<sup>1</sup>, Herica Tertulino Domingues<sup>1</sup>, Leandro Alves Da Silva<sup>1</sup>, Lionel Segui Goncalves<sup>2</sup>

<sup>1</sup>Federal Rural University of the Semi-Arid, Mossoró, Rio Grande do Norte, Brazil.

<sup>2</sup>University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Extreme climatic factors, such as high temperatures and intense solar radiation, can affect thermoregulatory control performed by bees. The objective of this study was to evaluate how temperature control by bees occurs in different ranges of air temperature and solar radiation, in two different situations: bees in thermal comfort and bees in thermal stress, in the semiarid northeastern region of Brazil. Thermal comfort was provided through artificial shading of the hives to prevent the penetration of direct solar radiation, while the bees were exposed to thermal stress. For this, we used 12 Langstroth hives with 6 beehives installed under protective shade and 6 installed directly under the sun. Climatic variables were measured and correlated with factors inherent to the colonies, such as the internal temperature, ventilation activity, and water distribution inside the hive. When the air temperature remained between 26 and 27°C and average global solar radiation was 600W.m<sup>2</sup>, the bees in thermal comfort did not perform any thermoregulatory mechanism. In the same range, bees under thermal stress distributed water in the hive and performed high ventilation activity as a strategy to lower the internal temperature. When the air temperature increased, remaining between 28 and 30°C, and the average solar radiation increased to 1000 W.m<sup>2</sup>, the bees, even when in thermal comfort, began to cool the colony, recruiting few bees for ventilation activity. In this same range, bees under thermal stress, instead of maintaining high ventilation activity, decrease this recruitment and instead use water to wet the body surface to prevent the body from reaching lethal temperatures. When bees are in an environment that provides thermal comfort, thermoregulatory efforts to lower the internal temperature of the colony are much smaller, making temperature control much more efficient, and consequently reducing the productive, physiological, and reproductive damage that is observed when the bees are under heat stress.



### PP-066 [Bee Biology]

#### A genetic improvement program for the native honeybee of the Iberian Peninsula, *Apis mellifera iberiensis*

Egoitz Galartza<sup>1</sup>, Melanie Parejo<sup>2</sup>, Iratxe Zarrakonandia<sup>3</sup>, Andone Estonba<sup>2</sup>

<sup>1</sup>ERBEL, Iberian Bee (Erle Beltz) Breeding Association, Zaldibia, Spain

<sup>2</sup>Applied Genomics and Bioinformatics, University of the Basque Country (UPV/EHU), Leioa-Bilbao, Spain

<sup>3</sup>IKERBASQUE Basque Foundation for Science, Bilbao, Spain

The Iberian honeybee, *Apis mellifera iberiensis* is native to the Iberian Peninsula and is well adapted to it. Unfortunately, in the last decade, we have observed the use of foreign bees, a rising trend which puts the native bee at risk due to hybridization. To improve the characteristics of local bees and discourage the use of foreign bees, we have initiated a genetic improvement program to promote this native Iberian bee. We build on previous studies showing that native bees are better adapted to the local environment and have higher survival rates, but often lack characteristics that make them attractive to commercial beekeepers.

In the Basque Country (Spain), the breeders association ERBEL is leading a breeding program for the genetic improvement of various relevant traits of the Iberian bee (e.g. gentleness, hygienic behaviour, swarming, honey yield) based on the BLUP method. First, to control mating within the program we established a geographically isolated mating station with mass production of drones of known genetic background. The mating station is located in the Ataun valley (58,9 km<sup>2</sup>) surrounded by mountains to limit the entry of foreign drones, and thus ensure that the virgin queens are exclusively mated by drones with desirable genetic background/traits. Second, with the objective of maximizing the success of directed matings, we have deepened our knowledge on the reproductive biology of the Iberian honey bees by identifying the specific characteristics of the mating flights of Iberian queens (e.g. flight time, number of flights, duration of successful flights, ect), studied by direct observation in the field. We also located Drone Congregation Areas (DCA) across the valley. Finally, to evaluate the effectiveness of the mating station and decipher the contribution of each of the drone-producing colonies to the matings, we developed a SNP-based paternity assignment test. With these efforts, we aim to provide commercial and hobbyist beekeepers in the Iberian Peninsula with well-adapted populations to reduce disease burden and achieve sustainable economic success.

### PP-067

#### The super-organism of a Honey bee colony and its social immunity

Grai St Clair Rice

Bee Joy

A Honey bee colony is made up of a multitude of parts, and elements, each playing a role in this dynamic super-organism. These elements in turn contribute to the social immunity of the colony, which is essential for a healthy hive.

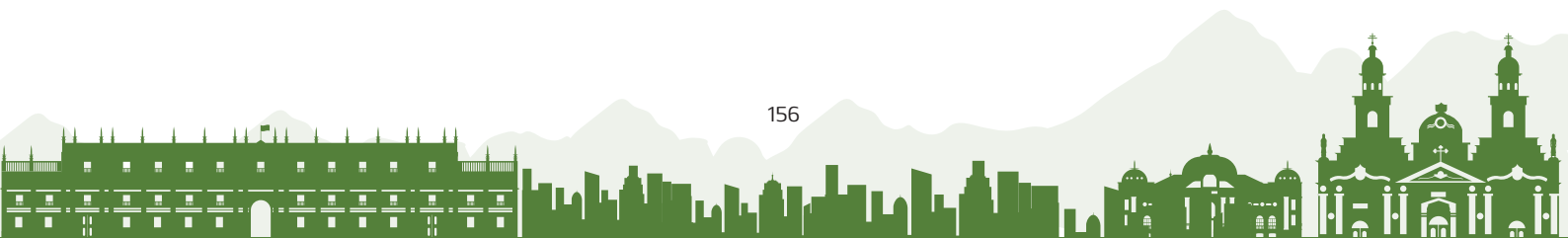
At the core of the super-organism are the castes of bees, each with their unique roles in the genetic diversity of the colony. The workers' age-based anatomy regulate their tasks according to sensory input and group feedback within the hive, including hygienic behavior and use of propolis.

Other core elements of the super-organism include: the beeswax comb, enzymes in the honey, microbes in the pollen, and the use of propolis for the nest envelop and other medicinal usage within the hive.

The microbiome of a colony includes the microbiome of each individual bee, as well as the entire internal environment of the hive.

Social immunity supports and reflects the super-organism within the hive. It is finely regulated to hopefully withstand pathogens and pests, and help maintain the homeostasis of the "body" of the super-organism.

Creating a poster that can visualize these dynamic relationships in an artful, fun and clear way is the goal. My visualization will not present new scientific research, however the poster will create a dynamic representation of elements of the super-organism of a Honey bee colony and its social immunity.





### PP-068

#### mtDNA melting analysis for quick characterization of Africanized honeybee *Apis mellifera* populations using honey samples

Leonardo Porrini, Pablo Revainera, Gregorio Fernandez De Landa, Pablo Gimenez Martinez, Edgardo Sarlo, Sergio Ruffinengo, Sandra Fuselli, Silvina Quintana

Centro de investigación en abejas sociales (CIAS), Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Mar del Plata (UNMDP). Centro de asociación simple CIC PBA. Mar del Plata, Buenos Aires, Argentina.

Honey contains mitochondrial DNA (mtDNA) from bees that produce it and can be used to identify their evolutionary origin, maternal lineage, or even subspecies. The mtDNA variation in *Apis mellifera* has been detected by different molecular methods (RFLPs, PCR-RFLPs, sequencing) and was previously used to characterize Africanized populations. High-resolution melting (HRM) analysis is a new technique that allows rapid identification of sequence polymorphisms in short amplicons generated by qPCR. The aim of this work is to identify the African mitotype in *Apis mellifera* populations by applying HRM. Honey samples were collected from 111 apiaries in 13 Argentine provinces between 2012 and 2018. Total DNA was extracted from sediment obtained by centrifugation of 10 g of honey and then subjected to qPCR in a final volume of 20 µl using a fluorescent intercalator and primers (APIS -F/Afr207R) that amplify a 207Pb product that allows discrimination between European and African origin. Positive controls (European/African) were used to corroborate qPCR product sizes and fusion curves. In addition, some products were purified and sequenced to confirm mitochondrial haplotypes. Only 26 honey samples from apiaries above 35° S in the provinces of Misiones, La Rioja, Catamarca, Formosa, Corrientes, Córdoba and Santa Fé showed an Africanized mitotype. The remaining apiaries below this border, mainly in the provinces of Buenos Aires, Rio Negro, Neuquén, and Entre Ríos, showed a European mitotype. The results are consistent with previous characterizations and with the distribution limit of Africanized populations in Argentina. Likewise, HRM analysis showed high sensitivity and effectiveness in mitotype characterization, as it requires very little DNA and requires only a single amplification reaction, reducing the cost and effort of sampling. In addition, it can be readily used in genetic selection programs in transition regions between European and Africanized populations, as it allows the identification of genetic resources of honey bee colonies based on the honey they produce.

### PP-069

#### Informative STR markers for identifying genetic characteristics of Ukrainian steppe bees

Tetyana Vasylykivska<sup>1</sup>, Olena Metlytska<sup>2</sup>, Sergiy Korinnyi<sup>3</sup>, Anatoliy Kharkovenko<sup>1</sup>

<sup>1</sup>NGO Brotherhood of Ukrainian Beekeepers, Lviv, Ukraine

<sup>2</sup>Poltava scientific research forensic center of the MIA of Ukraine, Poltava, Ukraine

<sup>3</sup>Poltava State Agrarian University, Poltava, Ukraine

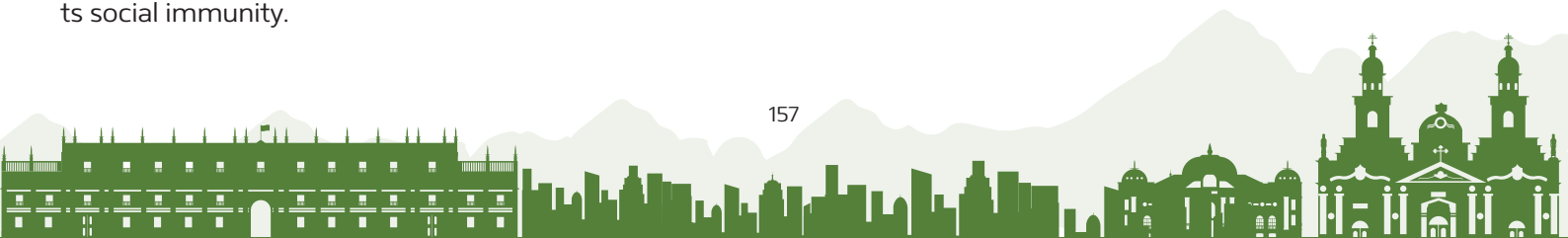
The preservation of the gene pool of Ukrainian steppe bees has recently become a significant problem due to their uncontrolled crossbreeding with gray mountainous Caucasians, Carniolans, and Buckfasts. This phenomenon leads to the loss of unique gene complexes of Ukrainian steppe bees, associated with increased adaptability to the specific conditions of the steppe and forest-steppe zones of Ukraine, with their unique floristic spectrum and continental climate.

One of the methodological approaches to preserving the gene pool of the autochthonous subspecies of bees is a set of selection-genetic measures aimed at developing identification and selectively significant molecular-genetic markers, creating genetic reserves of Ukrainian steppe bees, and combining methods of classical and marker-associated selection for their main productivity traits.

The purpose of our own research was to create highly informative STR markers for determining the population and individual ethological characteristics of Ukrainian steppe bees.

We analyzed the primary DNA sequences (*Apis mellifera*) from the GenBank database: DNA AJ509537, AJ509586, and AF140070 and constructed our own designed primers using the FastPCR ver 6.1 program. We selected the optimal conditions for amplification to combine the primers in a multiplex system. When analyzing the population of Ukrainian steppe bees (village of Machukhy, Poltava region), a total of 5 alleles were detected in each locus with levels of actual heterozygosity ranging from 0.667 to 0.917 for AJ509537 and AF140070, respectively. After carrying out an ethological experiment to determine the level of aggressive response to an external stimulus using a classical method modified by us, working bees were selected for further determination of their affiliation with genetic patrilineages using the developed STR markers. The scientific hypothesis of the predominant influence of the parental genotype (drone contribution) on the degree of defensive behaviour expression is confirmed, which can be used in breeding programs aimed at reducing the aggressiveness of the Ukrainian steppe bee.

ts social immunity.



### PP-070

#### Conservation status of *Apis mellifera ruttneri* inferred from whole genome sequences

M Alice Pinto<sup>1</sup>, Carlos Ariel Yadro<sup>1</sup>, Dora Henriques<sup>1</sup>, Marion Zammit Mangion<sup>2</sup>, Thomas Galea<sup>3</sup>, Dylan Farrugia<sup>1</sup>, Giovanni Cilia<sup>4</sup>, Noureddine Adjlane<sup>5</sup>, José Rufino<sup>6</sup>, Antonio Nanetti<sup>4</sup>, Raquel Martín Hernández<sup>7</sup>

<sup>1</sup>CIMO, SUSTEC, Instituto Politécnico de Bragança, Portugal

<sup>2</sup>Department of Physiology and Biochemistry, University of Malta, Msida, Malta

<sup>3</sup>Breeds of Origin Conservancy, 63, Nerik Sant, Haz-Zebbug, Malta

<sup>4</sup>CREA- AA, Bologna, Italy

<sup>5</sup>UMBB-DZ, Bourmedes, Algeria

<sup>6</sup>CeDRI, SUSTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>7</sup>CIAPA-IRIAF, Marchamalo, Spain

*Apis mellifera ruttneri* is the native honey bee subspecies from the Maltese islands (Malta, Comino and Gozo). This African-lineage subspecies is adapted to the harsh environmental conditions and to the limited forage season of these islands. For many years, it was considered highly hybridized, due to the large and sustained importation of foreign subspecies, especially *A. m. ligustica*, perceived by many beekeepers as superior or for commercial reasons. Recent studies based on morphometry and mitochondrial DNA have suggested the occurrence of *A. m. ruttneri*, particularly in the main island. Conservation initiatives and a breeding program have been developed, but to date its conservation status is not fully known. As part of the MEDIBEES project, here, over 50 *A. m. ruttneri* georeferenced colonies collected from across Malta, as well as 50 several reference subspecies (*A. m. ligustica*, *A. m. siciliana*, *A. m. iberiensis*, *A. m. sahariensis*, and *A. m. intermissa*) were whole genome sequenced. The population structure and admixture were assessed from genome-wide single nucleotide polymorphisms using model and distance based-methods. The results show varying levels of admixture proportions of *A. m. ruttneri* with C-lineage honey bees across Malta but also a shared ancestry with the honey bees of north Africa, consistent with the putative natural colonization from Africa in ancient times.

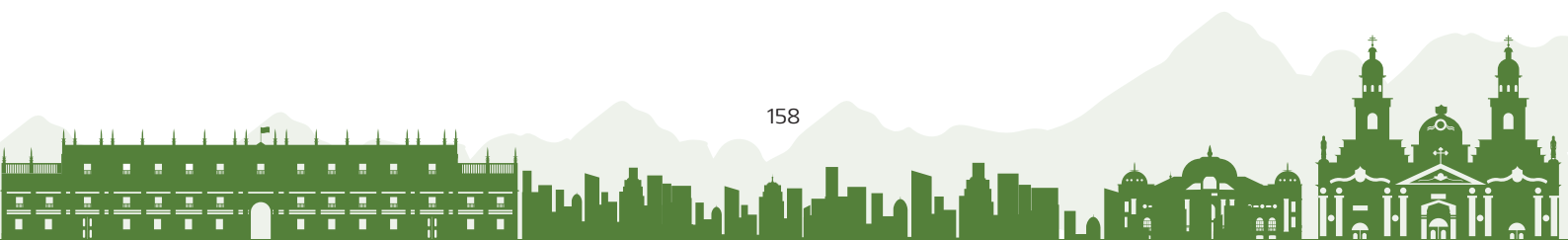
### PP-071 [Bee Biology]

#### Modeling the effects of honey bee (*Apis mellifera*) premature self-removal behavior on colony survival

Jordan Twombly Ellis, Juliana Rangel

Department of Entomology Texas A&M University

Honey bees are currently being challenged by multiple biotic and environmental factors, many of which act concomitantly to affect colony health and productivity. One negative effect of stress is accelerated age polyethism wherein stressed bees perform tasks at a younger age than healthy bees. Mathematical models of accelerated age polyethism have demonstrated that colonies that exhibit accelerated foraging ultimately collapse. We recently documented an extreme example of accelerated age polyethism, a behavior we are calling premature self-removal. General developmental stress was shown to cause honey bees to remove themselves from the colony before they could fly, leading them to die prematurely. In this study we modeled this premature self-removal behavior to determine the effects of self-removal on colony survival. In order to do this, parameters from previous honey bee models were used to inform our base model. Onto this we added stress-driven self-removal behavior. We found that premature self-removal has the potential to accelerate colony collapse and trigger higher rates of the similarly detrimental precocious foraging behavior. More extreme stress was found to lead to higher rates of self-removal. We found the stress point at which there is a high enough rate of self-removal and precocious foraging to ultimately lead to colony collapse. This will be beneficial in informing beekeepers of management practices in regard to stress levels such as mite thresholds and pesticide contamination points past which colony collapse is likely.



### PP-072

#### Crude protein and in vitro digestibility of the protein supplement PROSISE® and its effect on *Apis mellifera* population and laying in commercial hives

Yhann Vallenás-Sánchez<sup>1</sup>, Víctor Benicio Valdivia Camargo<sup>3</sup>, Cesar Eduardo Honorio Javes<sup>1</sup>, Wilson Castillo Soto<sup>2</sup>

<sup>1</sup>CAPDES, Department of Agronomy and Zootechnics, National University of Trujillo, Trujillo, Perú.

<sup>2</sup>Universidad Privada Antenor Orrego, Trujillo, Perú

<sup>3</sup>Importaciones Pecuarias S.A.C., Arequipa, Perú.

The honeybee (*Apis mellifera* L.) plays an important role in food and human health. However, climate change and loss of natural habitat reduce the amount and variety of pollen. This affects posture, bee population, and production performance. For this reason, artificial diets are used to reduce the negative impact of pollen shortage. The objective of the study was to evaluate the effect of an artificial diet with high digestibility on the posture and population of commercial hives. According to the authors, this is the first study to evaluate the digestibility of an artificial diet. An artificial diet was formulated with Animal-Space® software using brewer's yeast, soy protein isolate, whey, standard poly floral pollen, vitamin and mineral premix, probiotics, L-Tryptophan, L-Valine, sugar syrup, and corn oil. In addition, protein level was evaluated by the Kjeldahl method (AOAC 984.13) and digestibility by the pepsin method (AOAC 971.09). For the field study, 16 commercial hives installed in poly floral crops (mainly corn and avocado) were chosen. The field study was carried out in Laredo-La Libertad at 600 meters above sea level. These colonies received two treatments during 23 days (T0: without protein supplement and T1: with 250g/15 days PROSISA® protein supplement). Both treatments received two Wangs® strips (40mg tau fluvalinate/strip) as varroasis prevention. Our results showed 41.0 % crude protein and 95.3 % digestibility for the PROSISA® supplement in meal, and 23.0 % crude protein and 91.9 % digestibility for the paste supplement. Also, PROSISA® significantly increased ( $p < 0.01$ ) the number of frames with brood (6.0 frames) and bee population (11.0 frames) compared to the control treatment colonies (4.0 and 7.0 frames, respectively). We conclude that the use of amino acids in the artificial diet increases digestibility, and protein supplementation increases the laying and population of commercial hives. We recommend evaluating supplements with different protein levels and their digestibility.

### PP-073

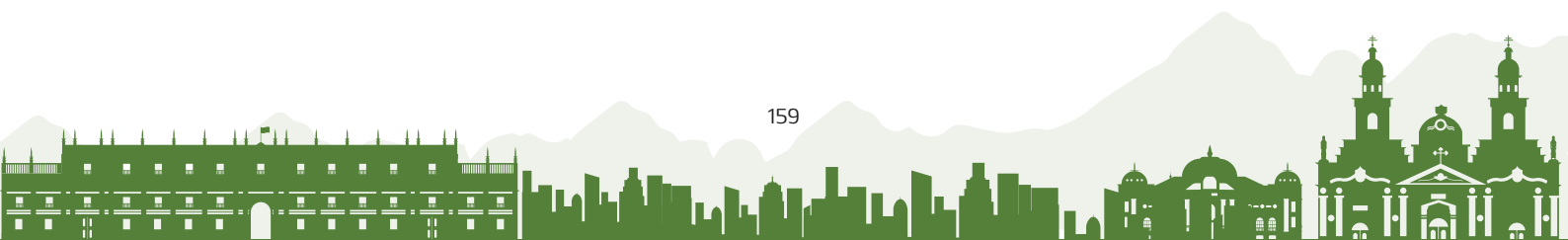
#### Using image deep learning to measure flight speeds and patterns of honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) near their hives

Kyeong Yong Lee, Sankar Kathannan, Young Bo Lee

Department of Agricultural Biology, The National Institute of Agricultural Science, RDA, Wanju, 55365, Republic of Korea

Bee traffic at the hive entrance can be used as an important indicator of foraging activity. We investigated the flight speed and patterns of honeybees and bumblebees near their hives as a basis for calculating bee traffic using the image deep learning. The flying speed of bumblebees ( $0.48 \pm 0.36$  m/s) near the hive was 1.4 times faster than that of honeybees ( $0.35 \pm 0.21$  m/s). The flight speed of honeybee leaving the hive ( $0.54 \pm 0.33$  m/s) was 1.7 times faster than that when entering the beehive ( $0.32 \pm 0.18$  m/s). Distance from the hive and flight speed showed a positive correlation (honeybee  $r = 0.600$ , bumblebee 0.659), and a significant linear regression model was derived (honeybee  $R^2 = 0.516$ , bumblebee 0.433). The flight pattern near the hive differed significantly according to bee at entering and leaving the hive. Honeybees mainly showed flight that changed flight direction more than once (69.5%), whereas bumblebees mainly performed straight flight (48.7%) or had a single turn (36.5%) in flight. When the bees entered the hive, honeybees mainly showed a one-turn or two-turn flight pattern (88.5%), and bumblebees showed a one-turn (48.0%). Whereas, when leaving the hive, honeybees mainly showed a straight flight pattern (63.0%), and bumblebees mainly showed a straight or one-turn (90.5%). There was a significant difference in flight speed according to the flight pattern. The speed of straight flight ( $0.89 \pm 0.47$  m/s) was 1.5 to 2.1 times faster than flight where direction changed. In summary, the speed and pattern of bees returning to or leaving the hive were different to from to the hive, and there were also differences between bee species. Therefore, our results can help determine the ideal frame rate for effectively capturing and recognizing the flying image of bees when calculating bee traffic by image deep learning.

Acknowledgments: This work was supported by a grant from the National Institute of Agricultural Sciences (Project No.: PJ016197012023).



### PP-074

#### Evaluation of reference genes for real-time quantitative PCR analysis from bumble bees (*Bombus terrestris*) of different

Sankar Kathannan, Young Bo Lee, Kyeong Yong Lee

Department of Agricultural Biology, The National Institute of Agricultural Science, RDA, Wanju, 55365, Republic of Korea

Bumble bees are important alternative pollinators and model insects due to their highly developed sociality and colony management. To better understand their molecular mechanisms, studies focusing on the genetic and molecular aspects of their development and behavior are needed. Although quantitative real-time polymerase chain reaction (qRT-PCR) can be used to quantify the relative expression of target genes, internal reference genes (which are stably expressed across different lines and tissues) must first be identified to ensure the accurate normalization of target genes. To contribute to molecular studies on bumble bees, we used *Bombus terrestris* to determine the expression stability of eight reference genes ( $\beta$ -actin (ACT), Arginine Kinase (AK), Phospholipase A2 (PLA2), Elongation factor 1 alpha (EF-1), Ribosomal proteins (S5, S18, S28) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) in five different lines and several tissues (ovary, thorax, fat body, and head) using RT-qPCR procedures and four analysis programs (RefFinder, NormFinder, BestKeeper, and geNorm). In general, the S28, S5, and S18 ribosomal protein genes and the PLA2 and EF-1 genes showed the highest stability and were therefore identified as suitable reference genes for the bumble bee species and their defined lines and tissues. Our results also emphasized the need to evaluate the stability of candidate reference genes for any differently designed lines and tissue conditions in bumble bee species.

Acknowledgments: This work was supported by a grant from the National Institute of Agricultural Sciences (Project No.: PJ015878032023).

### PP-075

#### Comparison of volatile compounds emitted by drone and worker brood of *Apis mellifera* infested by *Varroa destructor* mites

María Lujan Corzo<sup>1</sup>, Macarena Muniente<sup>1</sup>, Romina Maria Russo<sup>1</sup>, Alberto Galindo Cardona<sup>2</sup>, Ana Carolina Monmany Garzia<sup>3</sup>, Maria Alejandra Palacio<sup>4</sup>, Alejandra Carla Scannapieco<sup>1</sup>, María Clara Liendo<sup>1</sup>

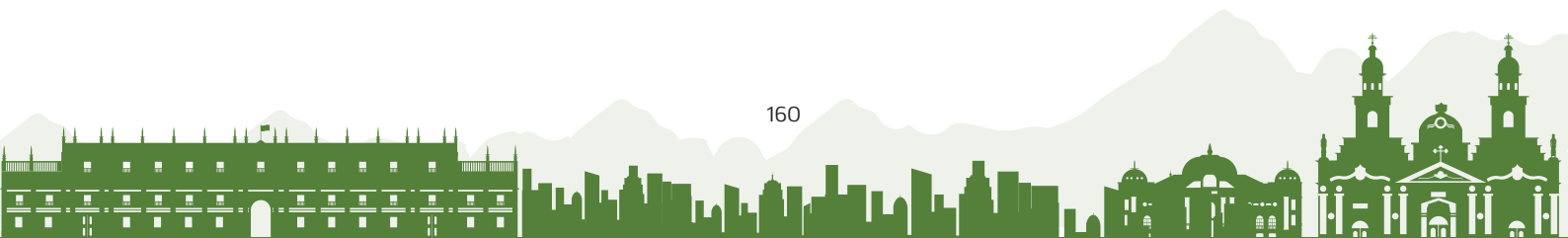
<sup>1</sup>Instituto de Genética "E. A. Favret", Instituto Nacional de Tecnología Agropecuaria (INTA)-Grupo vinculado al Instituto de Agrobiotecnología y Biología Molecular (IABIMO-CONICET)

<sup>2</sup>Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Entomología, Fundación Miguel Lillo, Tucumán, Argentina

<sup>3</sup>Instituto de Ecología Regional (Universidad Nacional de Tucumán-CONICET), Yerba Buena, Argentina

<sup>4</sup>Unidad Integrada INTA Balcarce - Universidad Nacional de Mar del Plata, Buenos Aires, Argentina

Hygienic behavior (HB) is a social health mechanism displayed by honey bee workers of *Apis mellifera* by which dead or diseased brood is removed from the colony. Such behavior is known to be induced by olfactory cues and has been described as associated with hygiene of brood cells parasitized by the mite *Varroa destructor*. Even though the mite has preference for drone brood cells, the efficiency of hygienic behavior toward this type of brood is significantly lower compared to worker brood. A possible explanation for this difference is that the compounds associated with infested drone brood are different from those emitted by infested worker brood, and hence, adult worker bees do not respond as efficiently. The aim of this research was to explore volatile compounds associated with dead or mite infested brood of drones and workers bees, to detect similarities or differences in chemical profiles. To this end, 20 pupae of each treatment were placed in glass aeration chambers. Volatiles were collected for 24 h on filters containing adsorbent material, eluted with dichloromethane, and analysed by gas chromatography. The treatments were: dead worker brood, mite infested worker brood, dead drone brood, mite infested drone brood and healthy drone and worker brood (controls). The chemical profiles obtained differed between the treatments. Specifically, the mite-infested and dead brood share some compounds, while others were exclusive of infested brood. This pattern was similar between worker and drone pupae. When the compounds emitted by castes are compared, we found no similarities. In addition, a variation in the abundance of  $\beta$ -ocimene - a compound associated with HB- was found for brood of both castes, being higher for mite infested and dead pupae compared to control pupae. These results would indicate that there are specific/exclusive compounds of infested brood that differ between worker and drone brood. Further studies are needed to evaluate the role of  $\beta$ -ocimene and the other identified compounds in triggering the HB.



### PP-076

#### 'SafeAgroBee' and honey bee adaptation in local and non local environments under different managements systems

Filipi Janja<sup>1</sup>, Adjlane Noureddine<sup>2</sup>, Charistos Leonidas<sup>3</sup>, Kolega Šimun<sup>1</sup>, Kovačić Marin<sup>4</sup>, Requier Fabrice<sup>5</sup>, Riva Clémence<sup>5</sup>, Fani Hattina<sup>3</sup>

<sup>1</sup>Department of Ecology, Agronomy and Aquaculture, University of Zadar, Croatia

<sup>2</sup>Department of Agronomy, Faculty of Sciences, University of Boumerdes, Algeria

<sup>3</sup>Department of Apiculture, Institute of Animal Science, ELGO 'DIMITRA', Nea Moudania, Greece

<sup>4</sup>Dept. of Animal Production and Biotechnology, Faculty of Agrobiotechnical Sciences Osijek, Croatia

<sup>5</sup>Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, 91198, Gif-sur-Yvette, France

The overall objective of the PRIMA project 'SafeAgroBee' is to contribute to adaptation and mitigation of the effects of climate change and other drivers negatively influencing the sustainability and the resilience of the agricultural system in the Mediterranean basin, ensuring the income of farmers and beekeepers through the food security. Under the umbrella of the 'SafeAgroBee' the adaptation of specific honey bee local populations from Algeria, Croatia, France and Greece are tested in their area of origin as well as in a second area of the same country, with different environmental conditions, and under a conventional or organic management system. The year-round performance of the populations, as well as the prevalence of diseases and parasites is of a major importance. The same populations are tested against SMR and REC behavior to identify any indicators of possible resistance to varroa. Furthermore, the capacity of these same populations to cope with the lack or abundance of proteinic food supplements during dry seasons is also under investigation. In 'SafeAgroBee' we also access the climatic conditions (by collecting historical and recent data) and produce future scenarios (2041-2060 and 2081-2099). By evaluating the influence of climate on prevalence of diseases and honey bee queen performance we will produce guidelines to ensure the resilience of beekeeping in different climatic and pasture regions.

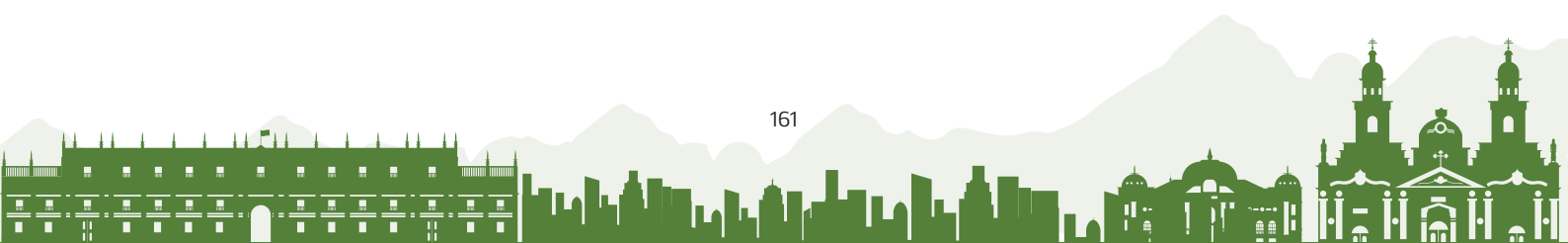
### PP-077

#### The Effect of Neonicotinoids on the Positive Transfer of Learning in the Visual Domain of Honeybees

Charisse Zou

Dougherty Valley High School, CA, United States of America

Honeybees serve as the world's most crucial pollinators and have a monumental impact on the agricultural industry of up to \$577 billion. However, their population is declining due to various factors, with pesticides being one of the most detrimental. This project investigated the effect of neonicotinoids imidacloprid and clothianidin on the positive transfer of learning in the visual domain of honeybees. Additionally, the project identified differences between varying dosages of these insecticides. Employing a self-constructed y-maze paradigm, three experiments were systematically designed including the control, imidacloprid, and clothianidin group. Experiments were conducted in a free-flying setting, simulating a real-world environment and eliminating external stressors. Each experiment consisted of training, in which bees were conditioned with the rule of delayed matching to sample, followed by a positive transfer test from pattern to color stimuli. Results of the control experiment indicated that positive transfer was successfully completed, as there were no significant differences in performance between training and testing ( $p=0.1453>0.05$ ). In imidacloprid experiments, it was concluded that medium-term memory was negatively impacted due to decreased transfer test performance ( $p<0.05$ ), while short and long-term memory remained intact. However, clothianidin experiments impacted both training and transfer tests ( $p<0.0001$ ). It had stronger effects than imidacloprid and it was concluded that long and likely short and medium-term memory were negatively impacted. The project emphasized the destructive effects of neonicotinoids on honeybees and stresses the need for change to avoid tremendous loss in the agricultural industry and environment.





## *Tropilaelaps mercedesae* parasitism changes behavior and gene expression in honey bee workers

Jing Gao<sup>1</sup>, Shilong Ma<sup>1</sup>, Shilong Ma<sup>2</sup>, Xinling Wang<sup>1</sup>, Yang Yang<sup>1</sup>, Qihua Luo<sup>3</sup>, Xing Wang<sup>4</sup>, Feng Liu<sup>5</sup>, Qiang Wang<sup>1</sup>, Zhongmin Fu<sup>2</sup>, Qingyun Diao<sup>1</sup>, Pingli Dai<sup>1</sup>

<sup>1</sup>Key Laboratory of Pollinating Insect Biology, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing 100093, China

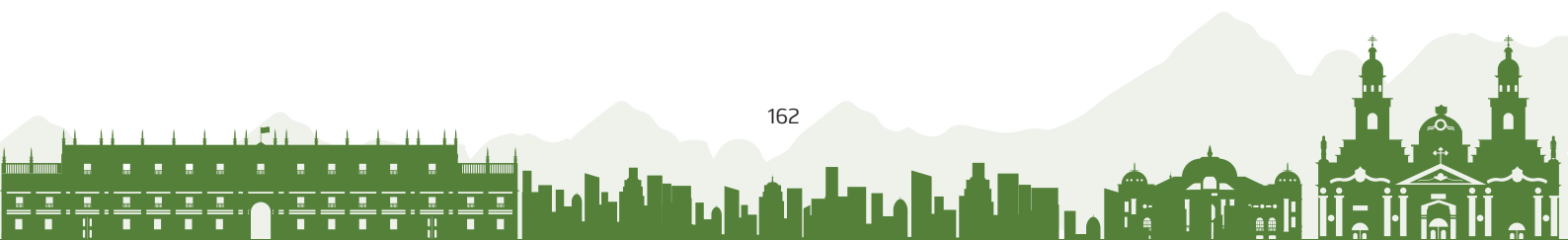
<sup>2</sup>College of Bee Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China

<sup>3</sup>Miyun Apicultural Station, Beijing 101500, China

<sup>4</sup>Beijing Apicultural Station, Beijing 100029, China

<sup>5</sup>Jiangxi Institute of Apicultural Research, Nanchang 330201, China

In recent decades, there has been serious concern about the decline of honey bees in the world. One of the most serious factors contributing to bee population declines is mite parasitism. Although *Varroa destructor* is the most widespread globally, *Tropilaelaps mercedesae* displays greater threat to bee colonies due to its smaller size, shorter phoretic phase, more rapid locomotion, as well as faster reproductive rate. *Tropilaelaps* can easily parasitize immature honey bees (larvae and pupae) and have both lethal and sublethal effects on the individual worker bees. Our study for the first time experimentally assessed the effects of *T. mercedesae* on olfactory learning, flight ability, homing ability as well as transcriptional changes in parasitized adult honey bees. *T. mercedesae* infestation had negative impacts on olfactory associated function, flight ability, and homing rate. The volume of the mushroom body significantly increased in infested honey bees, which may be correlated to the lower sucrose responsiveness as well as lower learning ability in the infested bees. The gene expression involved in immune systems and carbohydrate transport and metabolism were significantly different between infested bees and non-infested bees. Moreover, genes function in cell adhesion play an essential role in olfactory sensory in honey bees. Our findings provide a comprehensive understanding of European honey bees in response to *T. mercedesae* infestation, and could be used to further investigate the complex molecular mechanisms in honey bees under parasitic stress.



### PP-079

#### Status of the urban beekeeping in Latvia

Aleksejs Zacepins, Vitalijs Komasilovs, Armands Kviesis, Olvija Komasilova

Latvia University of Life Sciences and Technologies, Faculty of Information Technologies, Jelgava, Latvia

Global emphasis on sustainability and resilience as well as concerns about disappearing honeybees facilitates the wider adoption and implementation of urban beekeeping. The increased popularity of urban beekeeping has prompted municipalities and cities around the globe to start developing guidelines and regulations for promotion of this activity. However, little research has been conducted on the specific challenges and opportunities of urban beekeeping in Latvia. This study aimed to fill this gap by conducting a comprehensive analysis of the current state of urban beekeeping in Latvia and identifying key challenges and opportunities for its future development. Within this study authors are making the social study to analyse the citizens' awareness about the urban beekeeping topic and conclude about its popularity and development in Latvia. Online survey was conducted and 207 respondents shared their thoughts and participated in the questionnaire. 65.2% previously had heard or know about the urban beekeeping topic and almost  $\frac{3}{4}$  (74.9%) of the respondents have a positive attitude towards the development of urban beekeeping in their city. Survey helped to identify key risk factors and benefits of urban beekeeping and shed a light on citizens' doubts about this topic. The results showed that urban beekeeping in Latvia is still in its early stages, with a limited number of beekeepers and hives in urban areas. However, there is a growing interest in the practice among the general public and a growing awareness of the importance of bees for pollination and biodiversity. The main challenges identified were a lack of knowledge and training among beekeepers, limited access to suitable urban habitats, and negative perceptions of bees among some members of the public. Opportunities for future development include increasing awareness and education about the benefits of urban beekeeping, promoting the use of sustainable beekeeping practices, and creating more suitable urban habitats for bees. Overall, this study highlights the potential for urban beekeeping to contribute to the sustainable development of urban environments in Latvia.

### PP-080

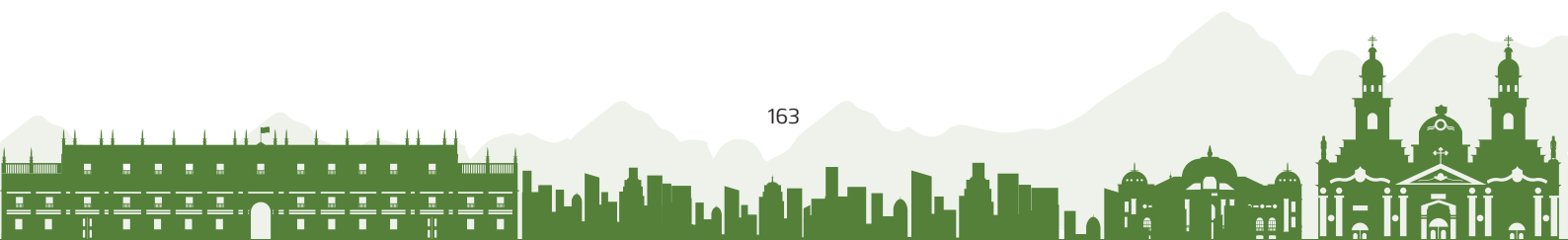
#### Status of the migratory beekeeping in Latvia

Aleksejs Zacepins<sup>1</sup>, Valters Brusbardis<sup>2</sup>, Olvija Komasilova<sup>1</sup>, Armands Kviesis<sup>1</sup>, Vitalijs Komasilovs<sup>1</sup>

<sup>1</sup>Latvia University of Life Sciences and Technologies, Faculty of Information Technologies

<sup>2</sup>Latvian Beekeeping Association

Migratory beekeeping is an approach to potentially increase the honey production, by moving bee colonies from one geographical location to another, based on the flowering times of the specific crops. As well, migratory approach can be applied for the pollination of the critical agricultural crops by providing the pollination service for the farmers. In relation to Latvia, previously there was no investigation of this topic and it is generally assumed that migratory beekeeping is not common for this country. Within this study authors are making the social study to analyse the Latvian beekeepers' awareness about the migratory beekeeping and its popularity in Latvia. Online survey was conducted and 237 respondents shared their thoughts and participated in the questionnaire. This research helped to identify key factors and benefits of migratory beekeeping and shed a light on beekeepers' doubts about this important topic. Main reason to use the migratory approach for the Latvian beekeepers is to diversify the assortment of honey. In other countries migratory approach is used by the beekeepers to provide a pollination service for the farmers, but in Latvia this approach also is not very common, as farmers are not very actively requesting the pollination service for their crops and there is no separate system to easily connect farmers with the beekeepers. Still in Latvia nectar crops are flowering in different periods of the active summer period and there is a possibility to increase the honey yield of the honeybee colonies by changing the foraging places, when nectar flow is finished in one location it potentially could be started in another location.



PP-081

### Identification of challenges and opportunities for formalization in the honey production chain in the State of Paraná, Brazil

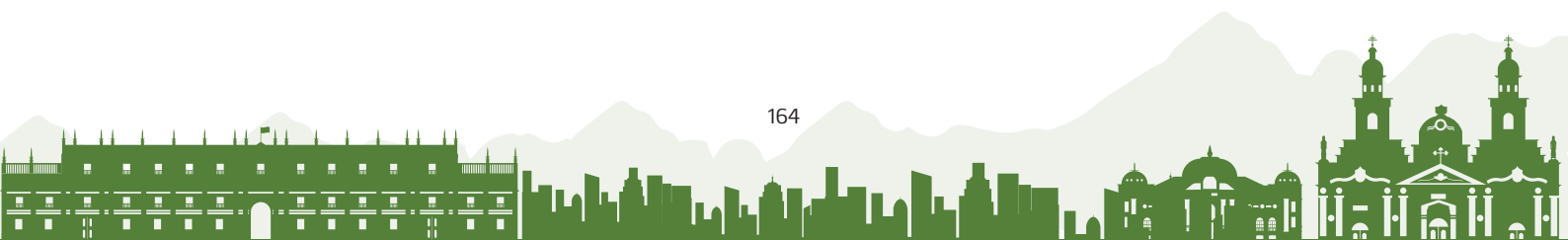
Eric Cezar De Camargo Walger<sup>1</sup>, Tuan Henrique Smielewski De Souza<sup>2</sup>, Tomaz Longhi Santos<sup>3</sup>, Joao Ricardo Dittrich<sup>1</sup>

<sup>1</sup>Postgraduate Program in Animal Science, Universidade Federal do Paraná, Brazil

<sup>2</sup>Postgraduate Program in Animal Science, Universidade Estadual de Maringá, Brazil

<sup>3</sup>Department of Animal Science, Universidade Federal do Paraná, Brazil

The bee production chains face a range of difficulties that limit their developments. These difficulties range from management techniques to organizational issues. Therefore, it is considered necessary to investigate the problems that limit the honey production, more specifically with regard to the formalization of this product, which can provide subsidies for actions to solve such problems. For this, online questionnaires were applied to 5 categories of actors of the production chain in the state of Paraná, Brazil. There were 32 responses from producers, 10 responses from representatives of associations, cooperatives or agroindustries, 9 responses from regulatory agencies, 9 responses from commercial establishments and 40 responses from consumers. The main challenge reported by the producers was the difficulty with the bureaucracy (68% of the answers), citing as a solution that the agro-industrialization norms are more adequate to the reality of the small and medium producer (64.5%). In this sense, representatives of associations, cooperatives and agro-industries declared that, in order to reduce informality in the honey production chain, it is necessary to re-elaborate the processing norms, taking into account the honey's specific characteristics and small and medium production (90%). On the other hand, 77.8% of the representatives of regulatory agencies consider the honey processing norms viable for beekeepers, including small and medium-sized ones. However, most of these representatives (44.4%) reported not knowing the details of beekeeping production. For the representatives of commercial establishments, the most important factor (88.9%) in the choice of a product to be commercialized is the presence of the inspection seal for products of animal origin, because for them it is difficult to trust the quality of honey brands (100%). Finally, 65% of consumers declared to prefer fully regulated products. These results highlight the importance of formalizing the production and processing of honey, on the other hand, reveals that producers, associations, cooperatives and agro-industries demand a revision of the current norms for the regulation of processing, something that is not compacted in the view of representatives of regulatory agencies.



#### First National Honey Contest of Uruguay

Gabriela Tamaño<sup>1</sup>, Selva Cora<sup>1</sup>, Rosana Díaz<sup>2</sup>, Italo Segredo<sup>3</sup>, Adriana Gambaro<sup>4</sup>, Laura Fariña<sup>5</sup>, Gloria Daners<sup>6</sup>, Natalia Arbulo<sup>7</sup>, Germán Azcune<sup>7</sup>, Liliana Vila<sup>8</sup>, Carolina Luzardo<sup>8</sup>, Victoria Figueredo<sup>8</sup>, Julio Sosa<sup>9</sup>, Belén Branchicella<sup>10</sup>, Laura Gurini<sup>11</sup>, Ana Castillos<sup>12</sup>, Inés Acosta<sup>13</sup>

<sup>1</sup>Laboratorio de Tecnología de miel y productos de la colmena (ApiUTEC), Universidad Tecnológica de Uruguay (UTEC), Paysandú, Uruguay

<sup>2</sup>Coordinación Honoraria del Comité Técnico, Rocha, Uruguay

<sup>3</sup>Rotary Club de Rocha, Rocha, Uruguay

<sup>4</sup>Área de Evaluación Sensorial, Facultad de Química, Universidad de la República, Montevideo, Uruguay

<sup>5</sup>Laboratorio de Biotecnología de Aromas, Facultad de Química, Universidad de la República, Montevideo, Uruguay

<sup>6</sup>Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

<sup>7</sup>Centro Universitario Regional del Este (CURE), Universidad de la República, Rocha, Uruguay

<sup>8</sup>Depto. de Análisis de Productos Agropecuarios, Laboratorio Tecnológico del Uruguay (LATU), Montevideo, Uruguay

<sup>9</sup>Depto. Desarrollo Local, Laboratorio Tecnológico del Uruguay (LATU), Montevideo, Uruguay

<sup>10</sup>Área Apicultura, Instituto Nacional de Investigación Agropecuaria (INIA), Colonia, Uruguay

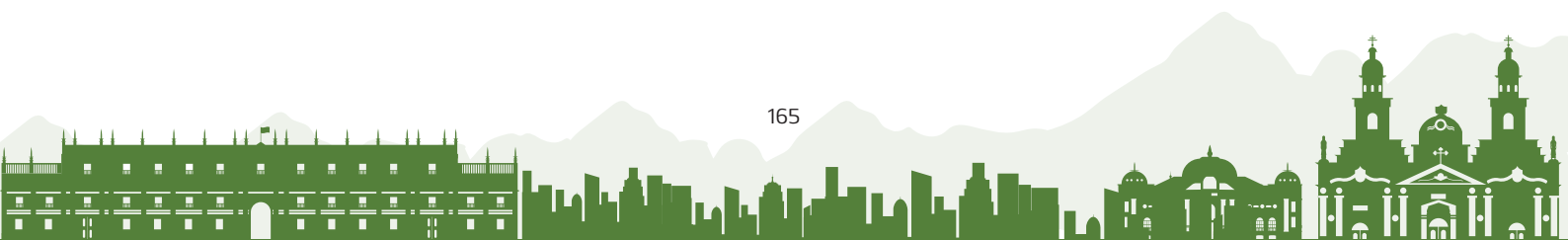
<sup>11</sup>INTA- PROAPI, Instituto Nacional de Tecnología Agropecuaria, Delta del Río Paraná, Argentina

<sup>12</sup>Desarrollo Rural, Intendencia de Rocha, Rocha, Uruguay

<sup>13</sup>Organización de Productores de Rocha, Rocha, Uruguay

Uruguay is a honey-producing country (13,000 tons/year), this being the most economically important hive product. 90-95% of honey produced is exported in bulk, without differentiation. Better sales prices and higher income for beekeepers can be obtained by marketing and exporting standardized. It is therefore one of the best ways to export and, indirectly, increase the internal consumption of Uruguay. The country requires comprehensive regulations and protocols that allow to have a letter of introduction for different honeys. Within this framework, at the initiative of Rocha Rotary Club, this inter-institutional project was created with the aim of disseminating the importance of beehive products, promoting the development of beekeeping and helping to position Uruguayan honey in the world and in the internal market. Thus an inter-institutional and interdisciplinary Technical Committee was established, with different areas of expertise in honey characterization: physicochemical, sensory, melissopalynological, volatile aspects, among others. The bases and organization for First National Honey Contest were developed, which took place at Rocha Agricultural Society from November 25 to 27, 2022, jointly holding exhibition and conferences. Participated 58 honeys from all over the country. A large audience passed through the Tasting Room evaluating the honey as consumers, following a specially designed protocol.

Real impact generated from this first action has been really positive, both with regard to the perception of the wide variety of honeys by consumers, as well as the appreciation of their own products by beekeepers. It constitutes a fundamental stone to the culture of differentiation of honeys as a tool to give them added value. It also marks a milestone at the national level, since for the first time scientific and technical institutions meet with a common objective that transcends institutional interests for the benefit of the country, proposing the constitution of a National Technical Committee for Honey Quality.



### PP-083

#### Why does save the bees mean save the planet?

Lucas Costa Cabral<sup>1</sup>, Wingly Santos Beltrame<sup>2</sup>, João Marcos Figueiredo Matano<sup>2</sup>, Nathalia Rodrigues da Silva de Carvalho<sup>2</sup>, Fernanda Giovana Martins de Oliveira<sup>2</sup>, Maria Claudia Colla Ruvolo Takasusuki<sup>2</sup>, Cristina Giatti Marques de Souza<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Universidade Estadual de Maringá, Maringá, Brazil

<sup>2</sup>Department of Biotechnology, Genetics and, Cell Biology, Universidade Estadual de Maringá, Maringá, Brazil

Research on scientific dissemination emphasizes the need to involve the population in socio-scientific issues, enabling it to act rationally. Therefore, the present study was developed for the population that is unaware of the factors and risks of the decrease in bee populations around the world and why this can affect all of us, human beings. In the light of rich previous work, we describe in a clear and didactic way the possible causes of the phenomenon of the disappearance of bees called Colony Collapse Disorder (CCD), which has worried many researchers, rural farmers, and beekeepers. In scientific opinion, the factors that most contribute to the decline of bees are the fragmentation of habitats, which originates in deforestation; parasites or pathogens that attack bees; and the use of pesticides in agricultural crops, which is the focus of our discussion. It is necessary for us to understand that humanity is deeply interconnected with the planet and the other forms of life that inhabit it, even if we pretend not to. Bee Movie, an animation released by DreamWorks in 2007, helps us understand the relationship between plants and animals, the pollination process, and the importance of pollinators, in addition to making the concept of teamwork very clear, mentioning the importance of small tasks to the realization of the whole. Therefore, we have to take better care of our surroundings, if not to preserve the beauty of nature, at least to guarantee our own survival. And general divulgation and awareness on this topic can help a lot, empowering the population to take action.

### PP-084

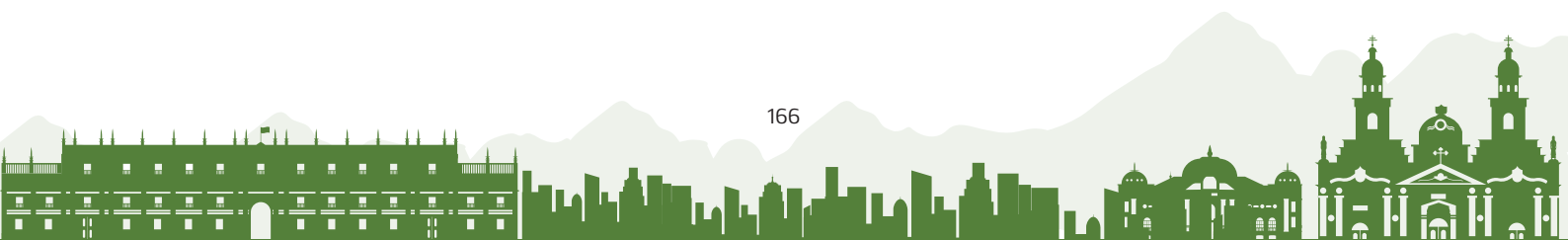
#### Development of the educational App, Bees in My Garden, as an innovative tool for learning about bees

Paola Hernández-Ching<sup>1</sup>, Rafael Calderon<sup>2</sup>, Beatriz Zepeda Ramírez<sup>1</sup>

<sup>1</sup>Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, Heredia, Costa Rica

<sup>2</sup>Programa Integrado de Patología Apícola, Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, Heredia, Costa Rica

The Tropical Beekeeping Research Center (CINAT) of Universidad Nacional, Costa Rica receives approximately 15 training requests on bees each year from mainly educational centers. Additionally, CINAT visits rural areas throughout the country where it is necessary to raise awareness among children about the importance and preservation of bees. However, the development of these activities requires a lot of logistics and adaptation of didactic material, which is a limitation for carrying out these activities permanently. Considering the above, the development of learning content on bees through a mobile application, called Bees in My Garden, was devised, which presents different topics dynamically and interactively, eliminates the spatial barrier involved in conducting face-to-face activities, and it also includes information on native stingless bees. The Design Thinking methodology was used to develop the application: empathize, define, ideate, prototype, and test. The problem to be addressed was analyzed, generating the thematic cores; then, the application prototype was developed, which was validated with the target population in an educational center (elementary school), allowing improvements to obtain the Minimum Viable Product (MVP). The thematic axes of the app were divided into: Bee Morphology, Main Contributions of Bees, Bees Face Dangers: Let's Learn About the Main Threats, and Hive Derivatives. *Apis mellifera* and *Asialia Tetragonisca angustula* are the two characters who, through interactive dialogues, present each game module and motivate participants to learn. Additionally, a teacher support guide was developed for learning about bees, based on the third thematic axis on main threats, with activities for classroom work. The application Bees in My Garden can be downloaded for free and is currently available in Google store for mobile devices with Android operating system.





### PP-085

#### “Market expansion by valuing honey products”

María José Torres, Gerardo Cárdenas, Diego Arias, José Donoso, Hamdy Zará, Marcelo Mardones

Asociación gremial hidromieleros de Chile

New market trends and the great challenges facing the beekeeping industry worldwide have led producers to seek new ways to diversify their supply and enhance the value of this precious raw material. One of the strategies with the best results at a general level is to use the competitive advantages and resources available to the companies, thus obtaining a higher profitability than the industry average. In this sense, one of the options proposed is to adapt the business model, achieving significant variations in the way activities are developed, allowing to value the resource, which in this case is honey.

This is where a product that, although it is relatively new in the market, it's considered the oldest alcoholic beverage of mankind, enters with great force. This alcoholic beverage, which ranges from 5 to 15 degrees, is traditionally known as mead. It is obtained by anaerobic fermentation of honey and water. This beverage has become one of the most promising honey by-products in recent years, not only because of its unparalleled flavor, but also because, depending on the production processes, it can preserve the functional and organoleptic properties of honey to a great extent. In this sense, the production and consumption of mead can help expand the beekeeping market by increasing the demand for honey and other products of the beekeeping chain. In addition, educating the public about the importance of bees and the need to support the local beekeeping industry as mead producers can inform consumers about the challenges faced by bees and how to support the beekeeping industry by buying local products.

### PP-086

#### Honey from European quality schemes in Slovenia - challenge and opportunity

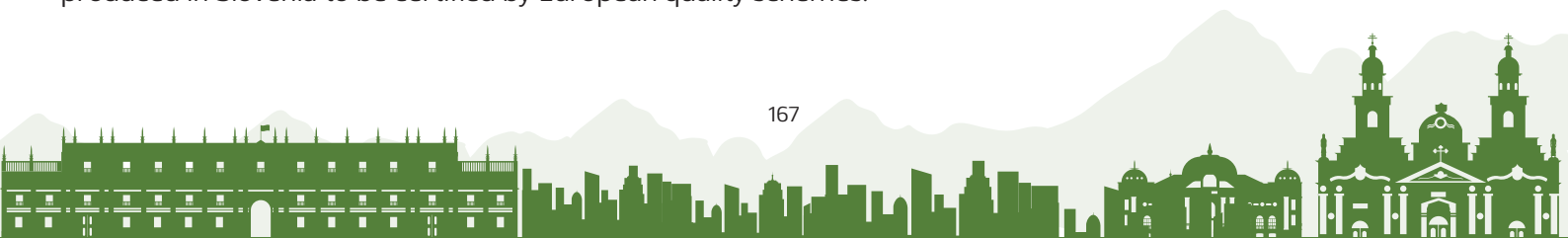
Tina Žerovnik

Slovenian Beekeepers' Association, Slovenia

Slovenia has three protected quality schemes for honey at EU level. It can be identified by a sticker on the lid of the jar, which includes the European quality symbol "Protected Geographical Indication" for Slovenian honey or "Protected Designation of Origin" for Kraški and Kočevski forest honey. Each sticker bears a serial number which identifies the producer and the place of production for each jar of honey.

Successful promotion and marketing of honey from schemes requires, on the one hand, raising awareness and encouraging beekeepers to join the scheme as producers. In this way, supply on the market is increased. On the other hand, it is important to increase the visibility and awareness of the customer of the benefits of buying this type of honey. It is important to raise awareness among beekeepers that the honey they produce is a high-quality product, due to its tradition and production method, which meets the European standards for certification in quality schemes. Improving and implementing marketing approaches with three key focuses: quality control, traceability and provenance.

The more beekeepers who enter schemes, the more honey of this type is on the market, which leads to a higher visibility of schemes by the customer and consequently a higher demand for this type of honey. This increases purchasing power, making it easier for honey to compete with other honeys on the market, while at the same time increasing added value and consequently improving the income situation of beekeepers. Through various European and national promotions as well as by advising beekeepers on how to improve the quality of honey production and the economics of selling honey, we are strengthening the supply of and demand for honey from schemes in Slovenia. The majority of honey produced in Slovenia meets higher standards than the European Honey Directive and meets the standards for inclusion in schemes. It is therefore essential that beekeepers participate in schemes to communicate to the consumer that honey produced in Slovenia is of a higher quality than the standard. The aim is for all honey produced in Slovenia to be certified by European quality schemes.



### PP-087

#### Teaching children the importance of consuming honey through talks and illustrated literature in Argentina

Veronica Maria Busch<sup>1</sup>, Diego Alberto Archaina<sup>2</sup>, Lucía Elisabet Brelis<sup>1</sup>, Florencia Campostrini<sup>2</sup>, Camila Carmona<sup>2</sup>, Andrea Carraza<sup>2</sup>, Lucía Ximena Centurión<sup>2</sup>, Maria Florencia Diaz<sup>1</sup>, Nancy Esposito<sup>1</sup>, Karen Irigoytia<sup>1</sup>, Juan Gabriel Nuñez Martinez<sup>1</sup>, Agustin Romani<sup>1</sup>, Giuliana Seling<sup>1</sup>

<sup>1</sup>Instituto de Ciencia y Tecnología de Alimentos de Entre Ríos, ICTAER UNER CONICET, Gualeguaychú, Argentina

<sup>2</sup>Facultad de Bromatología, Universidad Nacional de Entre Ríos, Gualeguaychú, Argentina

Socially it is known that the learning and habits acquired in childhood would affect decision-making such as the choice and preference of food for the whole life. In order to promote the consumption of honey and the appreciation of the honey bee a group of professors, researchers and students from the National University of Entre Ríos, Faculty of Bromatology (UNER Facultad de Bromatología) have done a Project title Mas abejas y toda la vida (More bees and for all life) since February 2022 until April 2023. In this project, a children's literature book was designed and colourful illustrated with flora (*Neltuma affinis*, *Butia yatay* and others) and natural landscapes from Entre Ríos, Argentina. This book tells the story of Api, a honey bee whose mission was to search for nectar, propolis, pollen or water. The members of this project gave many workshops to primary school children from 7 to 8 years old. Firstly they gave a talk about the differences in the types of honey bees (worker, queen and drone), the dance of the honey bee, the parts of the hive and the nutritional information of the hive products (honey, pollen, royal jelly, propolis). Then a game was made where the children played honey bees and collected flowers. The children could also put on beekeeping outfits and look and touch real hives. Finally, the Apibooks were given as a gift to each child in order to continue the activities at school and to promote home reading. With this work, hundreds of children have learnt the importance of caring for bees and the consumption of honey and other products from the hive.

### PP-088

#### Impacts of Climate Change on Beekeeping in Mediterranean Climates

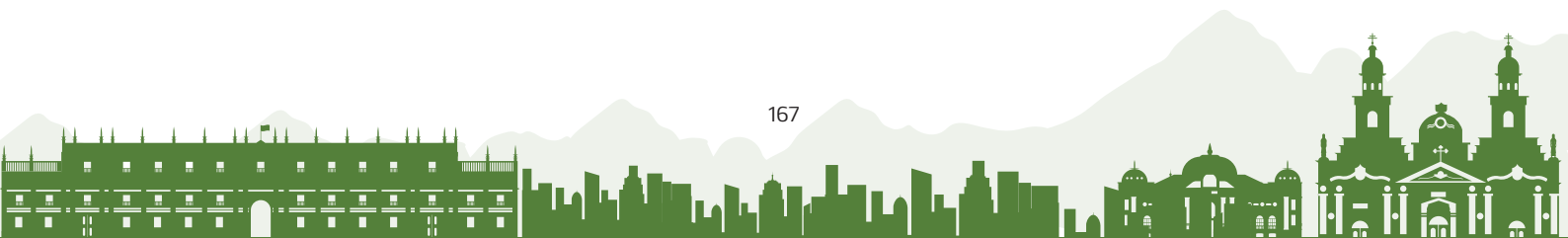
Martina Gajardo<sup>1</sup>, Ariel Muñoz<sup>1</sup>, Karen Yañez<sup>2</sup>, Ana Lopez<sup>1</sup>, Valentina Lepe<sup>3</sup>, Héctor Arrieta<sup>1</sup>, Enrique Rojas<sup>2</sup>, Germán Zapata-Hernández<sup>1</sup>, Caludia Fuentes<sup>1</sup>, Jessica Holthehuer<sup>1</sup>

<sup>1</sup>Centro de Acción Climática, Pontificia Universidad Católica de Valparaíso. Av. Brasil 2241, Valparaíso, Chile.

<sup>2</sup>USM Bee Lab, Universidad Técnica Federico Santa María. Av. España 1680, Valparaíso, Chile.

<sup>3</sup>Instituto de Conservación Biodiversidad y Territorio, Universidad Austral de Chile, Valdivia, Chile.

Climate change threatens the provision of ecosystem services from forests to society, especially in areas affected by drought. Chile is one of the countries most affected in Latin America by reduced rainfall and desertification. These changes have generated a great impact on water availability for communities and ecosystems, the latter appearing in vital processes such as growth and reproduction. In recent years, drought has generated important losses in forest vigor and changes in the abundance and seasonality of flowering, which has been especially marked in the central and south-central areas of the country, where a mainly Mediterranean climate prevails and the greatest honey production is generated. Recent studies have also shown that climate change is having negative consequences for honey production, which has led beekeepers to adopt adaptation measures to better cope with these impacts. In order to perform a comparative analysis between the different Mediterranean regions of the world, this study aims to assess the impacts that Climate Change is having on beekeeping and the different adaptation measures implemented by beekeepers in Mediterranean climates. For the development of this work, a bibliographic search was carried out and collaboration networks were established with Spain, France and Italy. So far, no such profound effects of climate change on beekeeping as in Chile have been identified, however, it seems important to develop these studies and to gather comparative information in different parts of the world, in order to advance in the field of adaptation. This study hopes to show the first results about the impact of Climate Change on Mediterranean beekeeping in the world.



PP-089

### Adulteration of bee honey: situational analysis in Costa Rica

Paola Hernández-Ching, Jeffrey Orozco-Barrantes, Keynor Ruiz-Mejias

Centro Internacional de Política Económica para el Desarrollo Sostenible (CINPE). Universidad Nacional.

The beneficial properties of bee honey for health and the trend towards natural foods have increased the demand for this product worldwide. However, the Food and Drug Administration (FDA) in the U.S. identifies bee honey as one of the main foods subject to food fraud, with the addition of sweeteners being the most commonly used method to reduce production costs and hence increase profit margins in sales. This affects both the economy of beekeepers, who cannot compete with prices in the market for an adulterated product, and potentially consumers, who may suffer health consequences due to the added ingredients. Costa Rica is not immune to this situation. With an annual production of 1,133 tons of bee honey in 2021, the country imported an additional 437 tons to meet the unsatisfied demand in the same period. The high consumption of this product has also led to the presence of products marketed as honey, characterized by a lack of labeling according to national regulations and a significantly lower price than the market average. In this context, the investigation covered the different actions that the country has taken to combat the adulteration of bee honey, using the quintuple helix innovation model: the measures implemented by different government organizations, the support provided by research centers to address this problem, the actions taken by the productive sector on their own, the role of consumers as direct stakeholders, and the environmental implications that a phenomenon like this can generate. It was evident that the country is not immune to the reality of the global situation regarding adulteration, and possible opportunities for improvement were presented to combat this problem, allowing for reflection by the various actors in the Costa Rican beekeeping system.

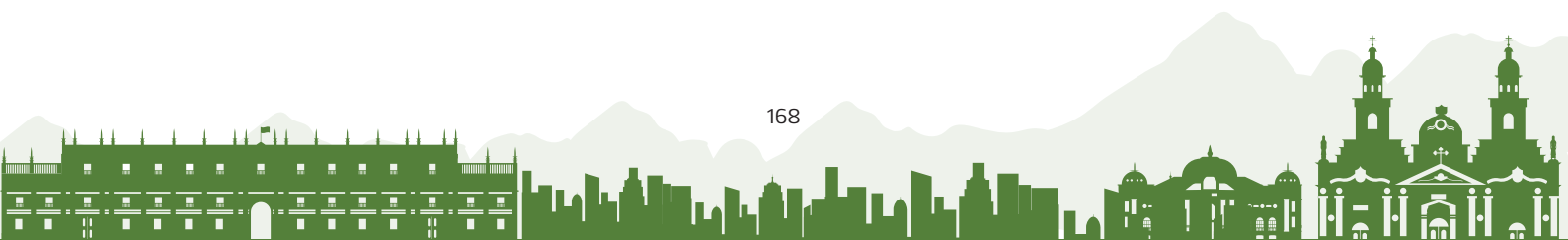
PP-090

### Modern teaching aids for training beekeepers and educating the wider public

Lidija Senič

Public Advisory Service for Beekeeping, Slovenian Beekeepers' Association, Brdo pri Lukovici, Slovenia.

The Slovenian Beekeeper's Association has for many years placed great emphasis on the education of beekeepers and raising awareness among the general public about beekeeping. To this end, it provides training, publishes educational and promotional materials, and develops new tools to help beekeepers solve problems and to bring the world of bees and their importance closer to the public. The Interactive Advisor is an online tool, accessible through a personal computer, tablet, smartphone, and designed to deepen specific knowledge in the field of beekeeping. It combines the use of different media with the inclusion of lectures, e-materials, e-tests and assessment. "Beekeeping VR" is a virtual reality beekeeping computer application using VR glasses. It gives an insight into the beehive, working with the colony, using beekeeping equipment, opening the hive and handling the honeycomb. When performing individual tasks, such as examining bee colonies, you are guided by a VR beekeeper. The Honey Story Pavilion is a mobile interactive beehive entirely designed both inside and outside as a classroom. It features beehives, special video content on screens in the honeycombs, equipment for honey production and storage, a honey tasting room, as well as tablet access to beekeeping resources. The history of beekeeping is shown through the painting of beehive panels and educational games to teach children about the life and importance of bees. The 3D Carniolan honey bee is a 1:100 scale replica of the worker bee *Apis mellifera carnica* measuring 170 cm in length and having a 90% morphological similarity to the real bee. It is set on a rotating platform, has moving body parts and educational animations. A 3D anatomical model of the Carniolan honey bee has also been created, showing 75 parts of the bee's body in longitudinal cross-section, from the interior and exterior. 360-degree beekeeping videos can be enjoyed with VR glasses, which give the user a sense of depth, quality, presence in space and three-dimensionality, plus a realistic portrayal of a particular beekeeping task. The films can also be viewed on a mobile phone, allowing you to watch them by moving your phone around in the room.



### PP-091

## Preliminary study on the application of the information technology system "ClassyFarm" to the apiary for the evaluation of honey bee colony welfare

Clara Montagnin<sup>1</sup>, Matteo Frasnelli<sup>1</sup>, Francesca Fusi<sup>1</sup>, Franco Mutinelli<sup>2</sup>, Giovanni Formato<sup>3</sup>, Cristina Roncoroni<sup>3</sup>, Paolo Bonilauri<sup>1</sup>, Luigi Bertocchi<sup>1</sup>, Valentina Lorenzi<sup>1</sup>

<sup>1</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna 'Bruno Ubertini' (IZSLER), Via Bianchi 7/9, 25124 Brescia (BS), Italy

<sup>2</sup>Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE), Viale dell'Università 10, 35020 Legnaro (PD), Italy

<sup>3</sup>Istituto Zooprofilattico Sperimentale del Lazio e della Toscana 'Mariano Aleandri' (IZSLT), via Appia Nuova, 1411, 00178 Roma (RM), Italy

ClassyFarm is the integrated information technology (IT) system of the Italian Ministry of Health for the risk categorization of livestock farms based on animal welfare (AW), biosecurity, antimicrobial usage and antimicrobial resistance. In ClassyFarm, the on-farm AW measurement is carried out by means of a multifactorial approach including both resource-based (RBIs) and animal-based indicators (ABIs). RBIs are input measures mostly related to farm management and housing factors, which can negatively or positively affect AW. ABIs are output measures for the assessment of the welfare consequences experienced by the animals, because of the exposure to management and environmental inputs. Each indicator is weighted according to its potential impact on AW, based on the EFSA guidance on risk assessment (2012). RBIs and ABIs are collected on-farm by trained veterinarians and then elaborated by a tailored algorithm to obtain risk scores. This study aimed to develop an AW assessment protocol applicable to honey bee (*Apis mellifera*) colonies by using potential valid, feasible and reliable RBIs and ABIs. For this purpose, sixteen Italian beekeeping experts, with different background, were involved into a focus group to discuss a list of RBIs and ABIs, previously selected by the authors through a review of the scientific literature. Despite the difficulties encountered related to the nature of honey bees and the limited availability of scientific knowledge about their welfare, 14 RBIs and 7 ABIs were identified as potentially able to assess the welfare of honey bee colonies. The proposed indicators will be weighted by an expert knowledge elicitation and an innovative algorithm for apiary welfare risk categorization will be created. The obtained protocol will be then tested on-field in Italian apiaries. This study sets the basis for a future application of the ClassyFarm system to the apiaries. The system will allow benchmarking of apiaries on an AW risk basis and could open the doors to a labelling system for honey bee products, that certifies the welfare level of the apiary in which they are produced. In addition, the implementation of this system could contribute to the protection of natural resources and biodiversity, promoting a more sustainable agricultural market.

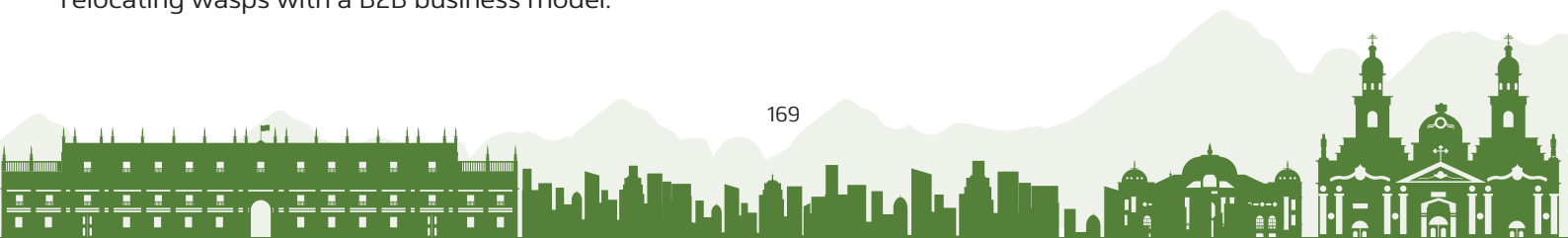
### PP-092

## Rescuing Africanized honey bees as part of a sustainable business model

Ilan Lechter

Apicultores y Rescatistas SAS BIC

Most of the times the activity of rescuing bees from unwanted places is not considered in the ecosystems of the apiculture or the honey business. Our company, Apicultores y Rescatistas SAS BIC started in February 2021 as a call to action from one corporate client (the third most important energy generator in Colombia) that after 2 years of looking for a company (not a person) to hire for business they couldn't find. Having working with bees for some time and with a corporate mentality we started the company with our entrepreneurial team in order to become the most recognized rescuers of bees and wasps in the American continent. Since the rescued bees will be relocate to apiaries the company is exploring the production of honey (and more products) and start the pollination division. Our goal with this presentation is to help our activity as rescuers become pivotal in the ecosystems where bees are considered. Governments, companies and people in general should help to promote our activity considering for example that in main 10 cities in Colombia there are around 36,000 anual reports to local authorities regarding bees and wasps. Our mission: Being the main pollinators of the planet and the most important natural pest controllers, Apicultores y Rescatistas SAS BIC's mission is to contribute to the sustainability of the world's food supply chain by rescuing bees and relocating wasps with a B2B business model.



## Beekeeping Economy

PP-093

### What is the main purpose of World Bee Day?

Peter Kozmus

Slovenian Beekeepers` Association

In 2017, at the initiative of the Republic of Slovenia, the United Nations declared 20 May as World Bee Day (WBD). Since then, we have celebrated WBD every year, but it is not just about celebrating. On this day, we try to inform the general public about bees and the challenges they face. It is extremely gratifying that every year more and more organizations that care about bees, nature in a broader sense and, ultimately, planet Earth, join in the celebration of the WBD. The annual celebration of the WBD by the FAO in cooperation with Apimondia also gives an important weight to the celebration. FAO is well aware of the great importance of bees and other pollinators for food production and therefore supported the 2017 WBD initiative. The main theme of the annual WBD is changing, but the three main objectives of the celebration remain the same: (A) to raise awareness about the importance of bees, (B) to inform people and especially decision-makers about the challenges facing bees and (C) what we can and must all do together in the future to ensure the survival of bees. The most important challenges facing bees vary from country to country, from region to region, so it is important to know them and to address them correctly in the context of the celebration and, above all, to find and present the right solutions. Without concrete solutions, we cannot help bees. In Slovenia, we recognized a few years ago that bees need better summer forage, so we decided to plant more than 2 million honey plants in the coming years. Another important focus on beekeeping is the Golden Bee Award, which is presented annually by the President of the Republic of Slovenia and is opened to people and organizations from all over the world. World Bee Day is a great opportunity to help bees, so let's use it to improve conditions for the survival of bees and other organisms. In doing so, we will be doing something good also for the Earth and ultimately for people.

PP-094

### Key challenges for honey traceability in Mexico, an experience from True Source Honey Certified

Héctor Luis Régules Garibay<sup>1</sup>, Courtney Alston<sup>2</sup>, Kate Dover<sup>2</sup>

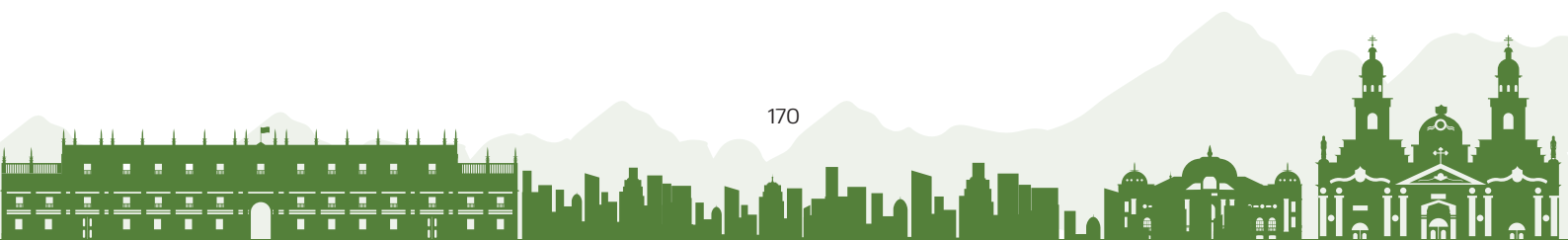
<sup>1</sup>Supply Chain, NSF de México

<sup>2</sup>Product Certification Audit Delivery, Global NSF International

True Source Honey Certified is a voluntary system for traceability and testing of authentic honey, where producers, processors, exporters and traders demonstrate, through a third-party audit, compliance with United States laws and international trades.

In the period of time from October 2020 to April 2023, a total of 25 Mexican honey processing and exporting companies have been evaluated into these requirements according to the "True Source Certified®" standard and checklists (versions 5.0, 6.0 and 7.1). This evaluation includes the inspection of facilities, review and testing of the traceability system, honey sampling, adulterated analysis, and an evaluation of company's suppliers.

After evaluating the audit reports, the following challenges the companies presents to develop and implement functional traceability systems are: 1) Development, evaluation and updating of a reliable, efficient and specific traceability system for the company; 2) Assignment and communication of the activities from responsible employees of implementation of the traceability system; 3) Training employees responsible for generating, implementing and evaluating the company's traceability and Recall system; 4) Identifying the loss of traceability as a result cause to classify a product as Non-Conforming; 5) Identifying potential risks for the loss traceability and generating the necessary corrective and preventive actions required to avoid them; 6) Generate efficient and reliable databases of the company's suppliers; 7) Advising to company's suppliers the implementation of efficient and reliable traceability systems and, 8) Development and implementation of product identification systems according to the types of company's processes.





### PP-095

#### Walloon and Brussels beekeeping (Belgium): Small sector and big expertise

Agnès Fayet, Sabine Malfait, Orianne Rollin

CARI, Beekeeping center of research and information, Louvain-la-Neuve, Belgium

Walloon and Brussels beekeeping has historical scientific and technical roots. From the end of the 19th century, Belgian beekeeping was a development model for European beekeeping. This « avant-garde » is still expressed today and is materialized by a network of mainly backyard beekeepers with strong expertise. This network is active, for example, in breeding, beekeepers' training, honey quality, hive products promotion, etc. Today, Walloon and Brussels beekeepers are getting organized to face the main challenges of our time: unpredictable climate variations, varroosis, asian hornet... The beekeeping sector in Wallonia and Brussels is not organized into economic production sectors but represents an important artisanal economy both from the point of view of production and pollination. The political powers have also been supporting it faithfully for several decades through support and development plans. Our poster aims to summarize both the anchoring of this regional beekeeping sector but also its organization and its development challenges.

### PP-096

#### Economic financial indicator for decision making in the beekeeping company

Roberto Saez<sup>1</sup>, Victor Rusas<sup>3</sup>, Javier Vagabculov<sup>3</sup>, Daniel Codutti<sup>2</sup>

<sup>1</sup>Instituto Nacional de Tecnología Agropecuaria, Universidad Nacional del Nordeste

<sup>2</sup>Cooperativa Apícola COPAP Ltda

<sup>3</sup>Instituto Nacional de Tecnología Agropecuaria

The present work arises from the problem of the beekeeping producers in “Estación Experimental Agropecuaria Colonia Benitez, from Instituto Nacional de Tecnología Agropecuaria (INTA), who manifest do not have systematized records of the activity and consequently do not have identified specifically the economic result or the financial flows of the activity.

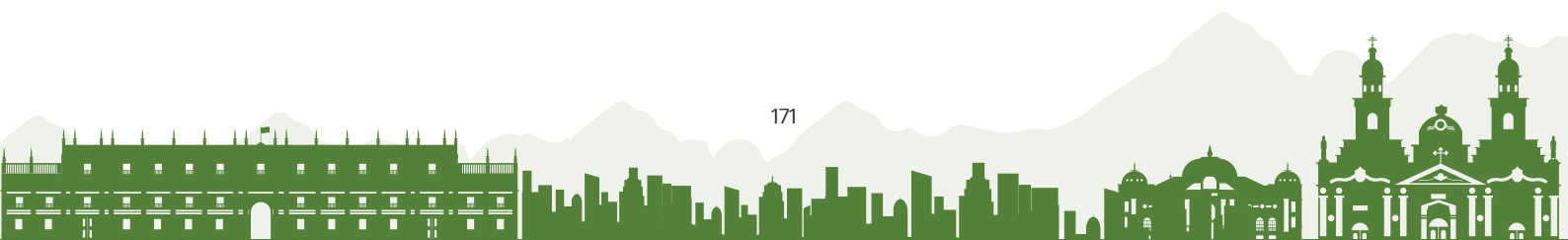
This problem clearly impacts both the activity in the area and the producer's decision-making, since those incipient producers and those who already have some experience in the activity, are unaware to get the gross margin of the activity and, consequently, show greater aversion to make investments in appropriate technology, either to improve their management or increase the number of hives.

Despite the fact that the economic income materializes when commercializing the honey, the production costs are not known, and it is commercialized at the price determined by the market, regardless of the production costs of each beekeeper. In this sense, it should be noted that each beekeeper will have different costs, since there are various factors that come together in the cost structure, such as distance to the apiary, number of hives, sanitary management, food, labor used, type of commercialization, performance of the area depending on the environment.

Taking into account the beekeeper problems raised, a systematized form of the activity as a proposal to obtain the Gross Margin and develop two financial indicators: VAN and TIR (Net Present Value and Internal Rate of Return). The gross margin is an interesting alternative, since it is an economic tool that is easy to use and implement, for decision-making and planning, in aspects related to the requirement of financial resources for the purchase of inputs throughout the production cycle, for mobility, health, supplementation, required labor, among others.

VAN and TIR indicators, allow to evaluate the investment in case of alternative projects and to determine which is the best investment; and the time required to recover the investment.

The objective is that the producer has adequate indicators for decision making.



## Beekeeping Economy

PP-097

### APlicité®, a national label for local authorities committed to bees and wild pollinators

Gabriel Peña

Union nationale de l'apiculture Française (UNAF), France

Protecting bees and wild pollinators represents a major challenge for humanity and our fellow citizens are becoming increasingly aware of this issue. This awareness is the result of decades of relentless advocacy by bee defenders.

Nowadays, many local authorities are getting involved in this process through concrete actions such as: requiring respect for biodiversity in public tenders, installing educational apiaries, or taking measures against pesticides. These actions reflect a strong social concern to environmental protection. Increasingly, for inhabitants a good living environment go hand in hand with a preserved nature. Honeybees taken as a communication medium are an outstanding symbol for local authorities in order to raise awareness among the population concerning biodiversity's threats.

UNAF believes that it is important to highlight local authorities' actions which benefit honeybees, that's why the APlicité label was launched in 2016.

APlicité's aim is to promote to a national scale these local initiatives in order to be generalised and at long term, intend to influence national politics.

In addition to being an official reward, the label is a real incentive to implement practices favouring honeybees and wild pollinators.

Once a year, an award committee made up of UNAF members and representatives of partner environmental organisations examine candidatures.

If a local authority deserves to be awarded, APlicité grants the candidature with 1,2 or 3 "Bees". Candidatures are studied depending on items: Sustainable development; Biodiversity; park/green space maintenance; Beekeeping; Environmental awareness.

Candidatures may come from all over France, from largest cities to small villages.

When the award committee deliberates, some advisements are given to local authorities in order to improve their environmental politics.

During the rest of the year APlicité's team continues advising and helping awarded towns and villages.

UNAF has already observed positive results on local policies thanks to APlicité. It represents an original way to bring together different interests and a combined action approach to defend honeybees and wild pollinators and Environnement.

PP-098

### "Let the bee fly" - Conscientization Action in Brazil by the Non-Profit Organization "Bee or not to Be"

Daniel Malusá Gonçalves<sup>1</sup>, Lionel Segui Gonçalves<sup>1</sup>, Rosane Peruchi<sup>1</sup>, Katia Gramacho<sup>1</sup>, Andresa Aparecida Berretta<sup>1</sup>, David De Jong<sup>1</sup>, Karin Rossi<sup>2</sup>, Silvia Bianchi<sup>2</sup>, Júnior Botrel<sup>2</sup>, Gustavo Almeida<sup>2</sup>, Palu Rossi<sup>3</sup>, Célia Queiroz<sup>3</sup>, Jonathan Soares<sup>3</sup>, José Paulo Garcia<sup>4</sup>, Gustavo Junqueira<sup>5</sup>, 6p Agency Team<sup>2</sup>, A Ilha Crossmídia Produtora Team<sup>3</sup>, Garcia Aviação Agrícola Team<sup>4</sup>, Conceito Assessoria Team<sup>5</sup>

<sup>1</sup>Bee or not to be - Non-profit Organization

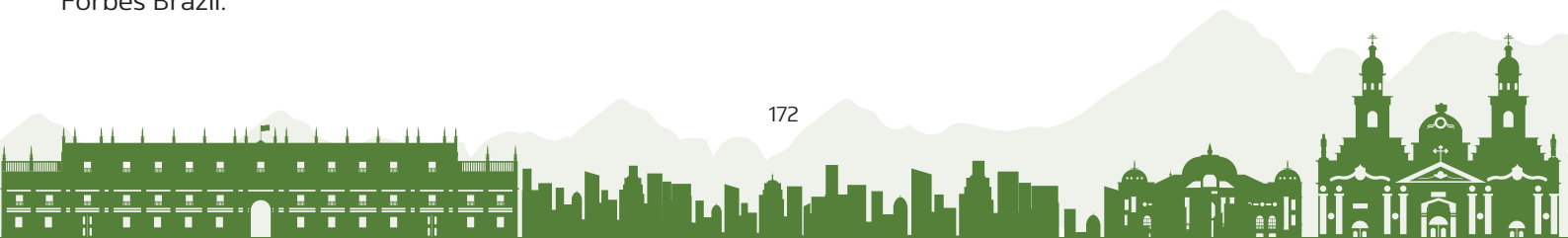
<sup>2</sup>6P Marketing & Advertising Agency

<sup>3</sup>A Ilha Crossmídia Produtora

<sup>4</sup>Garcia Aviação Agrícola

<sup>5</sup>Conceito Assessoria

Brazil has a great biodiversity of bees; more than 3000 species have been documented visiting flowers. They help guarantee the reproduction of plant species and ecosystem maintenance. Unfortunately, despite their importance, many millions of bees are constantly being killed due to various causes, including climate change, diseases, deforestation, and intensive and indiscriminate use of agrochemicals in agriculture, resulting in irreparable losses for the environment and for beekeeping. Brazil has a great agricultural vocation due to a productive model that favors a concentration of crops in large farms. However, as part of this scenario, pesticides are widely and frequently indiscriminately applied, often by plane, resulting in considerable risk of drift. Consequences include massive losses of bees and many beekeepers giving up their profession. The Association for the Protection of Bees "Bee or not to Be", located in Ribeirão Preto, SP, Brazil, in a region with a strong agricultural vocation, took advantage of the World Bee Day on May 20, 2022, to promote an unusual action that had great repercussions. "Bee or not to Be" transformed one of the airplanes normally used to apply pesticide into a "Giant Bee" that flew over fields and cities in the region of Ribeirão Preto, displaying the phrase: "Let the bee fly". This flight was conducted after a public breakfast that consisted mainly of products from plants pollinated by bees, showing images of the bees at work pollinating, with guests representing key stakeholders (beekeepers, producers, authorities, students, researchers, and other members of society) to show the importance of the bees and why it is necessary to promote the correct use of the agrochemicals to allow a harmonic coexistence between the bees and agriculture. The action also involved the preparation of a video that was launched in June 2023. The action was organized by the marketing agency 6P and the video maker company "A ilha crossmídia". The effects of the action were very impacting, resulting in 33 publications, including 28 websites, three TV reports, a newspaper section, and a radio program. Of special note was a publication about the event by Forbes Brazil.



### PP-099

#### Got your hives exposed to pesticides? What are your rights

Noa Simon Delso<sup>1</sup>, Charlotte Ehrmann<sup>1</sup>, Cindy Adolphe<sup>1</sup>, Alice Bernard<sup>2</sup>

<sup>1</sup>BeeLife European Beekeeping Coordination

<sup>2</sup>Client Earth Europe

It is well known that both pesticide residues and bees move freely in the environment, and sometimes they come across in higher or lower degrees. Depending on the level of exposure, these residues may generate bee die-offs, reducing the health and performance of the colonies or contaminating beekeeping products, not being able to sell them. In any case, beekeepers suffer the unexpected collateral damage of the activities of neighbour (or less neighbour) citizens. In many countries, the precautionary principle and polluter-payer approaches apply to the authorisation and use of pesticides. In the case of environmental contamination revealed by bees or beekeeping products, finding the individual that caused the contamination may be complicated. Furthermore, the pesticide authorisation process envisages to evaluate the environmental risk of exposure to one pesticide applied to one field. In real conditions, this is barely the case, and bees and humans are exposed to the summary of the different pesticides applied in our environment's many fields and surroundings. As a result, the level of exposure could be increased and mixed with other products. As a result of this reality, beekeepers may either lose their colonies or the marketing capacity of their products, which can see their content in pollutants go above Maximum Residue Levels established by authorities to preserve food safety. What can beekeepers do, then? What is the procedure to be compensated for these collateral damages? Can they be compensated for these collateral damages? The answer to this question is neither straightforward nor a one-solution-fit-all case. We present here a general approach, principles and action recommendations that beekeepers in each region must tailor to their situation.

### PP-100

#### General beekeeping practices and main stressors identified by beekeepers in the Mediterranean

Antonio Nanetti<sup>1</sup>, Raquel Martín Hernández<sup>2</sup>, Marion Zammit Mangion<sup>3</sup>, Maria Alice Pinto<sup>4</sup>, Dylan Farrugia<sup>3</sup>, Rachel Zammit Mangion<sup>3</sup>, Adjlane Noureddine<sup>5</sup>, Asmaa Anwar Eissa<sup>6</sup>, Giovanni Cilia<sup>1</sup>, Ahmad Yousef Daour<sup>7</sup>, Claudia Garrido<sup>8</sup>, Nizar Haddad<sup>9</sup>, Dora Henriques<sup>4</sup>, Chadi Hosri<sup>10</sup>, Mustafa Necati MUZ<sup>11</sup>, Soledad Sagastume<sup>2</sup>, Thomas Galea<sup>12</sup>, Medibeas Consortium<sup>13</sup>

<sup>1</sup>CREA-AA Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy

<sup>2</sup>CIAPA-IRIAF Centro de Investigacion Apícola de Marchamalo, Marchamalo, Spain

<sup>3</sup>UM Department of Physiology and Biochemistry, University of Malta, Msida, Malta

<sup>4</sup>CIMO Mountain Research Centre, Polytechnic Institute of Bragança, Campus de Sta. Apolónia, Bragança, Portugal

<sup>5</sup>UMBB-DZ M'Hamed Bougara University of Boumerdès, Faculty of Science, Boumerdès, Algeria

<sup>6</sup>Plant Protection Research Institute, Giemeza, Agricultural Research Center, Dokki, Giza, Egypt

<sup>7</sup>JBU Jordanian Beekeepers Union, Jordan

<sup>8</sup>BeeSafe, Hamm, Germany

<sup>9</sup>NARC National Agricultural Research Center, Amman, Jordan

<sup>10</sup>UL Lebanese University, Faculty of Agriculture, Beirut, Lebanon

<sup>11</sup>UNK University of Namik Kemal, Faculty of Veterinary Medicine, Tekirdağ, Turkey

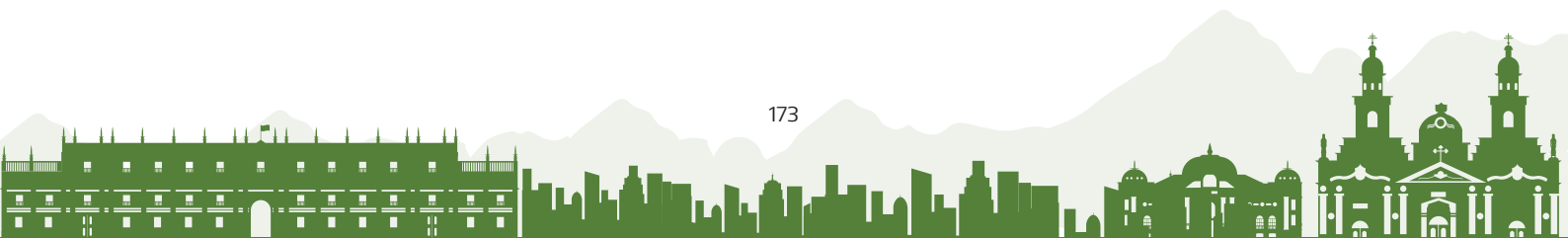
<sup>12</sup>Breeds of Origin Conservancy, Mosta, Malta

<sup>13</sup>Various institutions

Beekeeping is an ecologically and economically important activity in the Mediterranean that is increasingly under threat from a combination of factors. In the MEDIBEES (Monitoring the Mediterranean honey bee subspecies and their resilience to climate change for the improvement of sustainable agro-ecosystems) PRIMA project we aim to identify honey bees that show resilience to stressors associated with climate change. An important first step in our work was to establish baseline data of practices in the region through the administering of a questionnaire on the beekeeping practices. Over 1022 responses were received from beekeepers in Algeria, Egypt, Jordan, Italy, Lebanon, Malta, Spain, Portugal and Turkey.

Beekeeping was overwhelmingly male dominated (ratio 9:1) and practiced largely by the middle-aged (mainly between 40-50 years). The majority of beekeepers reported having 10-50 boxes with the exceptions of Algeria and Jordan where the majority reported keeping more than 100 hive boxes. Across the Mediterranean the Langstroth hive box was most commonly used. Despite the perception of migratory beekeeping being frequently practiced, only Lebanon and Turkey reported significant levels of transhumance (>50.0 and 70.0% of beekeepers respectively). 51.2% of beekeepers reported practicing queen rearing with the majority of these (51.4%) reporting re-queening their colonies every 2 years. Interestingly, the majority of all beekeepers agreed with the statement that their native honey bee is endangered where pesticide use, lack of adequate forage and parasite infestations such as the Varroa mite were repeatedly cited as causing the greatest losses to honey bees in general. Climate change and urbanisation were also linked with colony losses by causing habitat loss. Supplementary feeding was reported to be an integral part of beekeeping in this region, with beekeepers reporting using between 0-5kg, closely followed by 6-10 kg of additional sugars.

This project is part of the PRIMA programme supported by the European Union.



### PP-101

#### Carrying capacity of some agricultural crops and effect of distance

Ladeja Bohorič<sup>1</sup>, Clemence Riva<sup>2</sup>, Fabrice Requier<sup>2</sup>, Leonidas Charistos<sup>3</sup>, Marin Kovačić<sup>4</sup>, Zlatko Puškadija<sup>4</sup>, Bojan Stipešević<sup>4</sup>, Fani Hatjina<sup>2</sup>, Janez Prešern<sup>1</sup>

<sup>1</sup>Agricultural institute of Slovenia, Ljubljana, Slovenia

<sup>2</sup>IDEEV, UMR EGCE, Université Paris-Saclay, CNRS, IRD, Gif-sur-Yvette, France

<sup>3</sup>ELGO 'DIMITRA', Nea Moudania, Greece

<sup>4</sup>Fakultet agrobiotehničkih znanosti, Sveučilište Josipa Jurja Strossmayera, Osijek, Croatia

Within the scope of PRIMA SafeAgroBee, we have launched a set of field experiments to evaluate carrying capacity and impact of distance between the crop and the apiary. In common experiment design, two different crops (mustard and sunflower) were selected in three different countries and their area measured. On two locations in crops's neighbourhood an apiary was established and equipped with scales, one closer to the crop and the other one further away. The colonies at these two locations were evaluated for strength and mass change of the colonies was recorded for the two apiaries for the duration of the flow from the crop.

Regardless of the crop, colonies of the closer apiary seem to collect on average more nectar compared to more distant colonies. In case of mustard (Slovenia), one apiary with 10 colonies was placed directly in the crop and the other with another 10 colonies was about 1 km away on the flat terrain. During the flow it seemed that the one closer to the crop collected gained weight faster, reaching edge of +1.4 kg and giving some indication about the impact of distance from the source of flow. The second crop (black clover) started blooming closer to the distant apiary which at one point overtook the closer apiary in average mass change. Similarly in Greece, where the competition was performed on Sunflower. Closer apiary gained 2.6 kg more on average than distant apiary. However, in France the net gain was negative in both apiary, yet it seems that closer apiary lost weight at lower rate.

In fourth country, Croatia, several scales were placed under monitor hives within bigger apiaries close to crops with sunflower. The gain was not always greater in colonies with lesser distance, yet the information on neighbouring crops must still be augmented with crops areas and putative competing colonies.

### PP-102

#### IPM4Bees: Attitudes About Honey Bees and Pollinator-Friendly Practices - A Survey of Iowan Beekeepers, Farmers, and Landowners

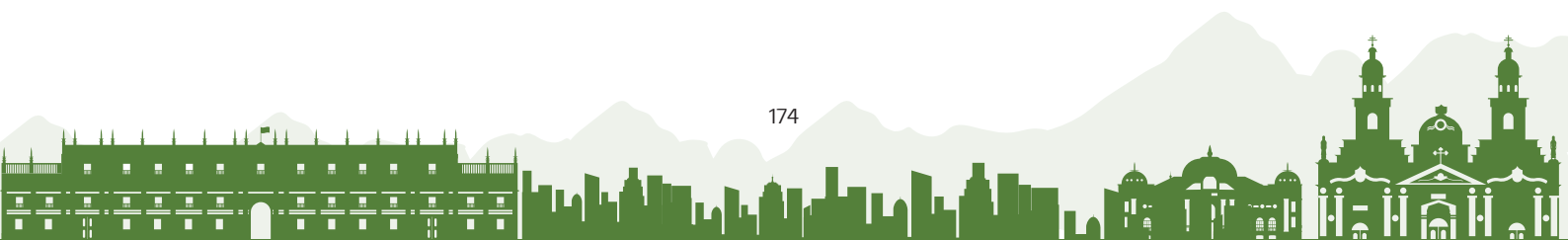
Randall Cass<sup>1</sup>, Erin Hodgson<sup>1</sup>, Matthew O'Neal<sup>1</sup>, Amy Toth<sup>1</sup>, Adam Dolezal<sup>2</sup>

<sup>1</sup>Department of Entomology, Iowa State University, Ames, Iowa, USA

<sup>2</sup>Department of Entomology, University of Illinois - Urbana Champaign, Illinois, USA

There are many challenges to maintaining healthy honey bee hives in the US state of Iowa where much of the landscape is dominated by agricultural production. Pollinator sustainability (i.e., wild bee conservation and sustainable beekeeping) relies upon management decisions by beekeepers, farmers, and landowners, but it is unclear if these stakeholders are aware of and use best practices. We present results of surveys administered to Iowan beekeepers, farmers, and landowners after extension programming on best management practices for sustaining honey bees (*Apis mellifera*) and other pollinators. Results show beekeepers, farmers, and landowners believe implementing pollinator-friendly practices is important. However, knowledge of tools to implement these practices, such as registering apiaries to avoid pesticide drift or cost-share programs for establishing habitat on their land, was very low.

Moreover, we discuss scaling this survey to a national level in the United States through the IPM4Bees Working Group, a collaborative organization made up of honey bee educators and extension professionals across the country. The group utilizes online survey techniques to collect data after extension presentations. Through this survey the IPM4Bees Working Group will gain insights on the major challenges beekeepers face in the US and develop new resources that are regionally relevant to beekeepers and other stakeholders.



## Beekeeping Economy

### PP-103

#### Sorting honeycombs to boost beekeeping enterprise turnover

Kodjo Logou Agossou

Multiplepurpose Research Production Station, Forestry and Beekeeping Arts Promotion Organization, Guerin-Kouka, Togo

It is surprising to see the honey wrapped on the combs according to the colors: the bees would have taken care to group the nectar samples according to a common belonging to the same category of vegetable essence. This natural predisposition is a situation that can help the beekeeper improve the profitability of his business. First of all, this makes it possible to offer many types of honey based on the colour, each with well-defined characters. As most of these properties are measurable, packaging labels will be precise as to the characteristics of the offer. Contracts will be more serious and less subject to misunderstanding. The beekeeper can thus obtain large honey demand contracts on the basis of a given parameter. In addition to supplying the pure products, it can supply a product resulting from mixing the latter but with varying proportions of its choice of such or such type of pure in the mixture. This increases the variability of its supply, which can extend between the extremes of pure products. This values the instinct of the bees to the delight of the beekeeper's turnover. In a second part, the beekeeper gains over the evolution of certain parameters, notably the visible ones such as the color, which are the first to affect the sale. If the bees separate the provisions, it can be assumed that it is for their best preservation therefore a more or less important stability of the characteristics. Since the change in characteristics is synonymous with alteration correlated with the reduction in turnover, stability must be sought: this is possible by sorting the honeycombs once outside the hive.

### PP-104

#### Beekeeping sector in the Scandinavian countries

Flemming Vejsnæs, Ole Kilpinen

Danish Beekeepers Association

The Apimondia congress will take place in the Scandinavian countries in September 2025, having the venue in Copenhagen. Therefore, it makes sense in this talk having a closer look into the beekeeping sector in the three organizing countries, Denmark, Sweden, and Norway. Driving distance from the southern Danish border, via Copenhagen over Stockholm to Kirkenes in Norway, is more than 2.500 km. This given a large variety in landscapes, from the intensive oilseed rape fields in Denmark, over the large forest areas in Sweden, to the large heather areas in Norway, before you end north of the polar circle. You see a large variety within the beekeeping sector, from the Buckfast dominated Denmark, to Sweden with a large proportion of ligustica bees, whereas in Norway the predominate bee race, is carnica. There are large protection efforts on the black mellifera bee, in all countries. With a population of more than twenty-one million people, the beekeeping sector is well organized with more than 21.000 members in 458 clubs. The commercial sector is relatively small with around 1 % commercial beekeepers. The commercial sector is under pressure of cheap imported honey and fake honey as well. It is difficult to keep a commercial honey production alive and, in that way, the pollination service is under pressure. In Denmark bee colonies for pollination in the clover seed production is imported from Germany. The high salary level and expensive living costs makes the economy even more tight. In this talk we dig deeper into the beekeeping sector in the three counties, looking on similarities, but mainly differences.

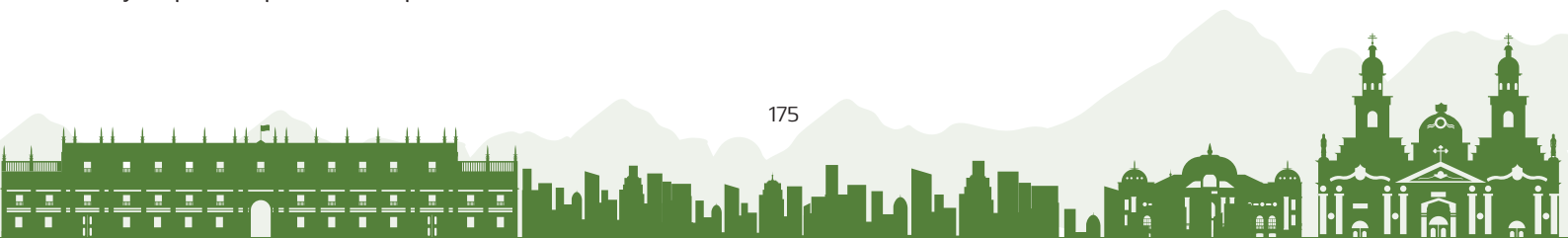
### PP-105

#### Future of European beekeeping: threats, challenges and perspectives

Róbert Chlebo

Slovak University of Agriculture, Faculty of Agrobiological and Food Resources, Tr. A. Hlinku 2, Nitra, Slovakia

Number of managed bee colonies in Europe is continuously growing (in EU 19 million + min. 6 million in other European regions). Most of colonies are kept by hobby beekeepers, total number of people involved in beekeeping is around 800 000. Review on main threats, challenges and perspectives covers following topics: Pests and diseases - approaches to treatment / prevention, invasive species, colony losses, low immunity, reduced immunity. Changes in climate and land use - bee colony nutrition. Pesticides and contaminants – agrochemicals, monocultures vs. good farming practice. Honey market - adulteration and profitability of beekeeping operations, low honey price, market volatility, legislative changes. Education, research and cooperation – young beekeepers' involvement, research and extension, Apimondia and other beekeeping federations activities. A preliminary assessment of the beekeeping year 2023 based on reports from some European countries will be presented. Actual situation of legislative changes related to beekeeping and honey import/export development in the EU will be discussed.





### PP-106

#### Development of sensory panels for the definition and recognition of honeys from the Mediterranean basin

Amelia V. González-Porto<sup>1</sup>, Gian Luigi Marcazzan<sup>2</sup>, Raquel Martín Hernández<sup>1</sup>

<sup>1</sup>Centro de Investigación Apícola y Agroambiental (CIAPA) de Marchamalo - Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRAF)

<sup>2</sup>Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria: Bologna, Italia, IT

The Mediterranean basin is characterized by various botanical species of plants from which the bees collect the nectar, which will give rise to a great variety of honey, both monofloral and polyfloral. A further source of variation in honey characteristics could be linked to the differences in floral preferences among the different subspecies of *A. mellifera*, but this aspect is not yet known.

All these factors influence the pollen spectrum and the physicochemical and sensory characteristics of honey. Sensory characteristics are fundamental in the honey market, as they are directly perceived by consumers and can influence appreciation and choice. However, consumers' preferences may vary according to their knowledge of the product and the different organoleptic and quality characteristics. It is therefore essential to study the organoleptic and quality characteristics of honey and to educate consumers. For this purpose, expert technicians must be trained to form and maintain a sensory panel whose task will be to describe, evaluate and promote the honey produced by the local bee subspecies. Both CIAPA and CREA have proven experience in performing sensory analysis as each institution has already developed sensorial panels. The alignment of the existing panels and the development of the new panels in the other partner countries will be led by CREA as already coordinator of the Sensory Analysis working group within the International Honey Commission (IHC). Under this supervision, sensory panels will be developed in all the participating countries (Italy, Spain, Portugal, Malta, Jordan, Algeria, Turkey) according to the MEDIBEES proposal (Monitoring the Mediterranean Honey Bee Subspecies and their Resilience to Climate Change for the Improvement of Sustainable Agro-Ecosystems), supported by PRIMA European funding.

In addition to the training of a panel of tasters at national level within each country, the trainers of each country carry out training activities on the different types of honey production in their environment, aimed at different audiences such as beekeepers, university students, consumers, in the villages as a point of support in rural development. More than a dozen of these activities have been carried out in the different partner countries with great success.

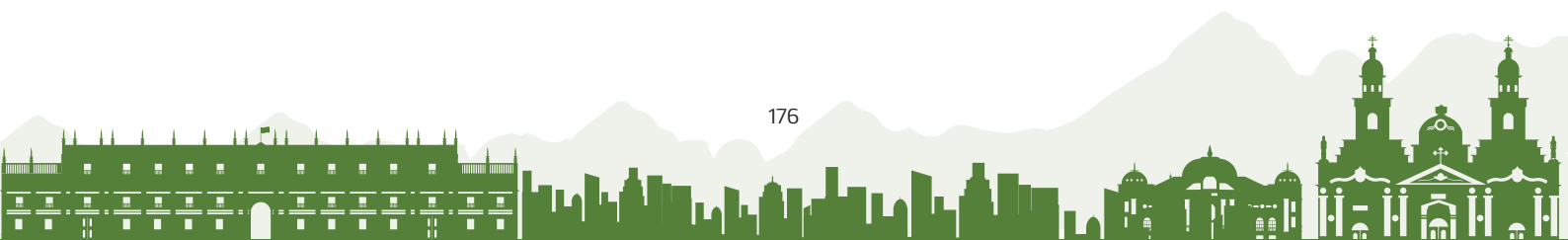
### PP-107

#### Situation of bee breeding in the Polloc village center, La Encañada, Cajamarca, Perú

Maivelin Claude Chunqui Cerquin, Alonso Vela Ahumada, Jhon Anthony Vergara Copacandori

Department of Agronomy, National University of Cajamarca, Cajamarca, Perú

"In the Population Center of Polloc, La Encañada district, province and department of Cajamarca, Peru, primary information was collected through surveys in order to determine the situation of beekeeping *Apis mellifera*". Surveys were applied to 50 beekeepers distributed in five villages. Thirty percent of the respondents were initiated in beekeeping through the implementation of a financed project and 70 % through self-learning; 80 % of the beekeepers have not been trained or technically advised, as opposed to 20 %. Seventy percent of the hives were installed from collected swarms and 30% correspond to core of bees acquired in the city of Cajamarca, predominantly of the Creole breed, but there are also crosses between Carniolan and Italian. A large number of hives (80%) are installed in agricultural ecosystems such as tarwi, corn and potatoes, while 20% have been installed in natural forests. All of them report that health problems include ants and varroa. Finally, honey production is 30.8 kg/hive/year (considering only 2 harvests per year) and is destined for the local market.



## Beekeeping Economy

### PP-108

#### BRAGGOT: "A drink without limits, you just have to dare to discover it"

Pablo Antonio E Maessen Bolla

Escuela Agrotécnica 4-025 Los Corralitos - Guaymallén - Mendoza

Those of us who venture into the production of meads and honey beers have a new way of innovating. The braggot drink that is waiting for its opportunity to become known in the beekeeping world, today the conditions are ripe to become the symbol of the strategic alliance between meads and artisan brewers, thus opening a new market both in the world of craft drinks as well as industrial drinks. The braggot is waiting for the occasion to resurface, despite the fact that its origin that goes back thousands of years ago, today has a new moment. It was a drink that had its heyday in medieval Europe and in the Renaissance, its name derives from the Irish and Welsh roots "branch" and "brag", used to refer to the "bud" of the cereal grains used for its preparation. In those times when drinking contaminated water was a symbol of disease, men found a way to quench their thirst, in these slightly acidic drinks with low concentrations of alcohol and flavored with honey. Probably the modern challenge lies in finding the exact ingredients in a balanced way for different palates and moments. Today the biggest challenge is not to repeat a medieval recipe, the challenge is to update the flavors with ingredients that we find in our current environment, if we look closely we will find a lot of creative potential in the use of barley, corn, quinoa, rice, and other cereals that are combined with the infinity of variants of honey and with ingredients from every part of the world.

The braggot can be drunk with abundant or calm foam, light or dark, fruity or astringent, with high or subtle alcohol content, there is truly something for all tastes. You just have to dare to discover.

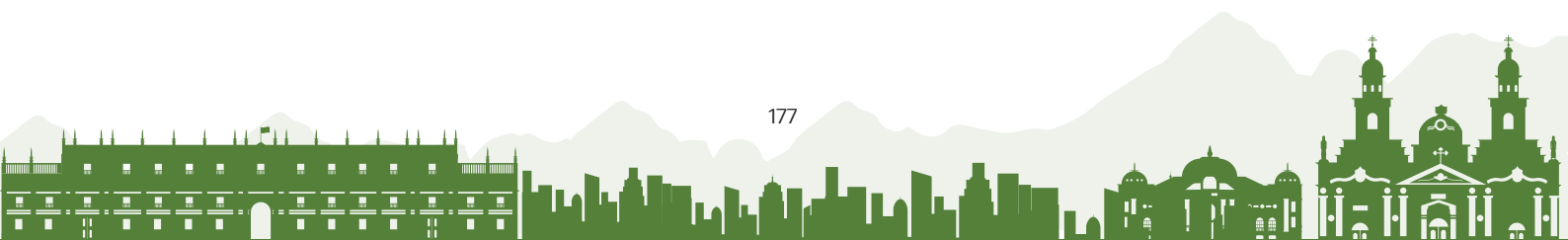
### PP-109

#### Honeydew honey in Uruguay: sources and characteristics

Estela Santos, *Ciro Invenizzi*

Universidad de la República, Faculty of Sciences, Ethology Section. Iguá 4225 Montevideo Uruguay

The chemical composition of honey depends on the floral or extra-floral origin used by bees and the botanical and geographical origin. *Apis mellifera* honey bees can produce honeys, based on extrafloral secretions from different parts of plants or excretions from insects. These sweet compounds that they collect are mixed inside the hive, with the nectar collected from the flowers or they can be stored in pure form if they are very abundant. In this case they take the name of honeydew honey. Currently the International Honey Commission defines the characteristics and quality parameters of different types of floral honeys, but does not have quality criteria for the different types of honeydew honey produced in the world. Therefore, characterizing the types of honeydew in each region is very relevant. In Uruguay, in recent surveys, the presence of honeydew in blossom honey has been related to native forest areas. In this study, field and laboratory records were reviewed to identify and describe sources of honeydew in Uruguay. And honeydews honey from apiaries were obtained to characterize them by melissopalynological analysis and analysis of physicochemical parameters (moisture content, electrical conductivity, color, Delta  $\delta^{13}C$  and F / G ratio Fructose / Glucose ratio, to authenticate the botanical origin of honeydew honey in this country. In the field trip records, observing the bees collecting sweet compounds, six food sources were identified for the bees that can be stored as honey. In different locations in Uruguay, bees were recorded sucking honeydew of *Epormenis cestri*, *Neocoelostoma xerophila*, *Melanaphis* sp. and *Cinara* sp excretion. There was also the collection of secretions of *Acacia logifolia* and *Xanthium spinosum*. The physicochemical characteristics of these honeydew showed differences that can be used as indicators of quality and origin. New studies must be carried out to better characterize each of the honeydew found.



## Beekeeping Economy

### PP-110

#### Importance of the production of mead for the training and technical education of new winemakers in the agrotechnical school of Los Corralitos in the province of Mendoza - Argentina

Pablo Antonio E Maessen Bolla

Escuela Agrotecnica4-025 Los Corralitos Guaymallén - Mendoza - Argentina

Currently the teaching of oenology in the technical schools of the province of Mendoza - Argentina begins with the knowledge of the cultivation of the vine and the management of the production of grapes to obtain different varieties of wines. Among these varieties, the renowned Malbec wine stands out, which has positioned this region to the east of the Andes mountain range as one of the main wine capitals worldwide. On the other hand, the agroecological conditions of the land and its altitude at more than 750 meters above sea level make this province a special place for the development of the wine industry recognized throughout the world. In the beekeeping environment, Argentine honey is also known for its volume and quality, being able to highlight that Mendoza honey is very important for its organoleptic characteristics typical of the flora of the semi-arid zone, as well as for its low moisture content, which makes it special for the production of meads.

Faced with this promising panorama, with very good raw materials of natural origin, as well as high-level knowledge related to obtaining fermented beverages, as well as technology for the preparation, production and fractionation of mead, it is important to teach new winemakers all the possibilities offered by the production of the different varieties of meads in their different alternatives going from the basic ones, made with only honey, or the sparkling ones, such as those that are produced with fruit, generating an infinity of proposals starting from the combinations called peymens with different varieties of grapes from *Vitis vinifera*. We are currently working thinking about the future within the classrooms of the agricultural school 4-025 of Los Corralitos to have new oenologists specialized in meads

### PP-111

#### Women in Argentine beekeeping

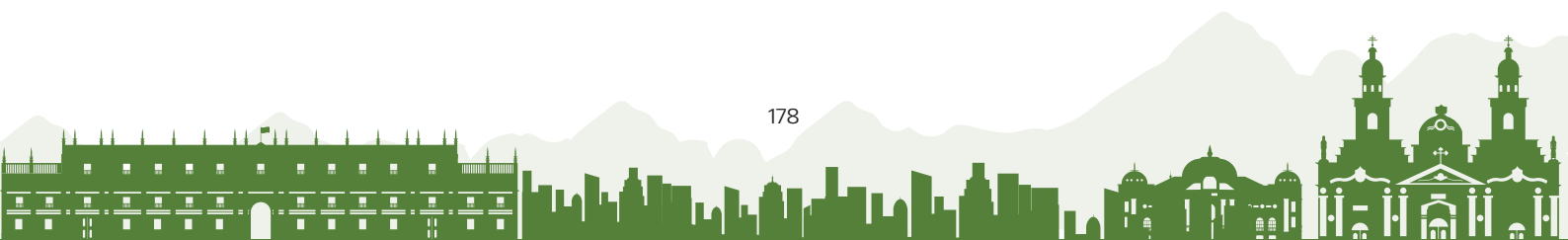
María Emilia Estrada<sup>1</sup>, Agostina Costantino<sup>2</sup>

<sup>1</sup>Departamento de Economía, Universidad Nacional del Sur; Laboratorio de Estudios Apícolas (LabEA - UNS - CIC)

<sup>2</sup>Departamento de Economía, Universidad Nacional del Sur; Instituto de Investigaciones Económicas y Sociales del Sur (IIESS - CONICET)

It is estimated that in Argentina, 10% of the people working in beekeeping are women.

Women's participation in beekeeping is usually linked to the need to supplement income at the farm level in rural areas, to the search for economic and social progress for their community, to small-scale beekeeping (due to supposed limitations in apiary management and access to land), and also to values more closely linked to the environment and ecology. Although there is some individualized dissemination of the participation of women in the production of honey and other beehive products, in the production of inputs, in their contribution to associative strategies and in contributions to scientific production, there is no academic research aimed at characterizing the women who carry out this activity at the national level. There are also no studies that allow us to understand their situation in terms of production and the social relations of which they form part. This work sought to characterize women who participate in the productive sector by means of a semi-structured survey and interviews with key actors in the sector.



## Beekeeping for Rural Development

### PP-112

#### Youths and Beekeeping Technology in Developing Countries

Victoria Onyekachi Odimba

Akanu Ibiam Federal Polytechnic Unwana Afikpo Ebonyi State Nigeria

Beekeeping for a long time has been in existence in Developing countries, from studies and observations needs more interests in specialty areas of the field such as Beekeeping technology. In this context technology means the science and art of making bee equipment which include hive, smoker, bee uniforms etc. Beekeeping in Developing countries may still be at its subsistence level. There is also the problem of sustainability and interest in this part of the world may be related to character and mindset sometimes. These motivated my putting up this piece. which is geared towards; giving an insight into the progress of youths in the Developing world in Beekeeping technology, also to highlight on the need for future progress to be planned using the youths. Beekeeping is a very lucrative venture that can help in boosting a nation's economy if done well. It is also essential as it can help in rural income generation, peri-urban and urban regions, this inclusively helps in sustainable job creation. Having all said and done, we can actually have a sustained Beekeeping by motivating the youths through educational training which may include workshops etc as youths are the future of tomorrow. Adoption of early grooming of youths in Beekeeping technology can help in having early youth development, sustainable entrepreneurship and other job creation.

### PP-113

#### Eco- beekeeping, the apiculture based on the nature of low costs and inputs

Alexander Garcia

Alexander García Molina, APIARIOS EL MIELOSO, CÚCUTA, Colombia

It is estimated that in Argentina, 10% of the people working in beekeeping are women.

Thinking in the development of the apiculture and the bees in the rural sector. We have developed a new concept based on love and respect of the laws of nature. What allows us the significant savings in economic resources, and at the same time prosperous and high harvest.

The ECO-BEEKEEPING is based on the LOVE – RESPECT and OBSERVATION. Which allows that any person to start with the bees without hard academy preparation. Instead, they can start with basic fundamentals that are easy to learn BASED ON THE NATURE.

In this order of ideas we present its main characteristics:

Location of empty hives (Langstroth, Layens, Exagonal, Kenia, and so on) at Tellurian crosses because bees are located according to these criteria. This allows the use of natural hives in the time of hiving and with the vigor of nature. However, it is important to continue following the natural laws.

Also, no stamped wax is used in the brood chamber and it is optional in the honey chamber, depending in the available resources.

In the ECO- BEEKEEPING no additional practice is performed in the honey extraction. In other words, it is only OPEN TO HARVEST. What allows less stress due to human intervention. Neither, we use additional accessories as excluding grid, marked or queen change, change of honeycombs of bees, and so on.

By experience and observation, the ECO- BEEKEEPING is related to the saving of time and resources, at least 70%, becoming the queen of the honey production of low inputs, achieving prosperous colonies with a 1% evasion with high harvest.

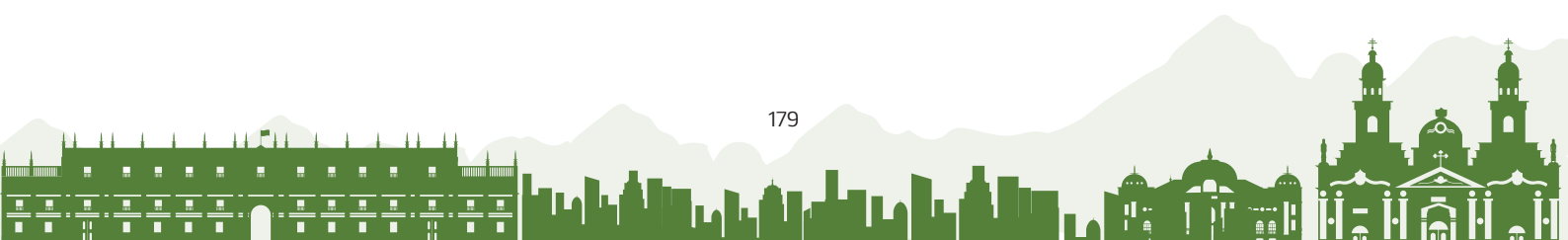
Besides, it is important to highlight that aggressiveness is reduced to its minimum biological, thanks to the reduction of ecobeekeeper visits.

The ECO- BEEKEEPING is definitely a successful way for the craft apiculture, based on the nature and low inputs.

Author:

Alexander García Molina

Apiarios el MIELOSO



## Beekeeping for Rural Development

### PP-112

#### Damaged but managed: the partnership between military veterans and honey bees in health recovery

Pamela Forgrave

Department of Cultural Studies, Trent University, Peterborough ON, Canada

In the liminal space between war and peace we encounter two subjects facing a health crisis: military veterans and *Apis mellifera*, the western honey bee. Advancements in science and technology in the past half century have resulted in fewer battle casualties and more veterans making the transition out of military life. However, identity challenges and mental health injuries have emerged as new crises in this population. At the same time honey bees are also in transition from being honey producers to pollinators working in the service of other crops. This shift, however, has led to a new pest and pesticide pressures, all of which are contemplated in the spectre of colony collapse disorder. Increasingly, efforts are underway to bring both groups together, in hopes that bees and veterans can help ease each other's challenges. Heroes to Hives, a veteran beekeeping program emerging in the USA, is one of the many initiatives seeking to support the mental and physical health of former and "six-legged soldiers" (Lockwood 2009). This paper explores how veterans understand their changing identity as they transition from a soldier to a beekeeper. It asks: what is to be made of their 'exceptional' citizen status when confronted with illegitimate violence in legitimized violent spaces? And what is in it for the honey bees? Drawing on insights from cultural studies and animal studies, I consider how identity is created by analysing the narratives of veteran beekeepers via websites, trade journals and podcasts. I show how ideals of protection are key to veteran beekeeping narratives, providing new ways of interacting with civilian spaces, while also reinforcing colonial and gendered understandings of nature at the same time. I therefore conclude that these veteran beekeeping programs may ultimately limit the forms of transitional care veterans and bees might find in one another and recommend an approach that makes a connection between human health and environmental health.

### PP-115

#### Empowering Rural Development through Medicinal Beekeeping and Apitherapy

Stefan Stangaciu

Romanian Society of Apitherapy, Phytotherapy and Aromatherapy

Many beekeepers primarily focus on producing and selling honey for food consumption.

While food is essential, it is not the only critical aspect of human health.

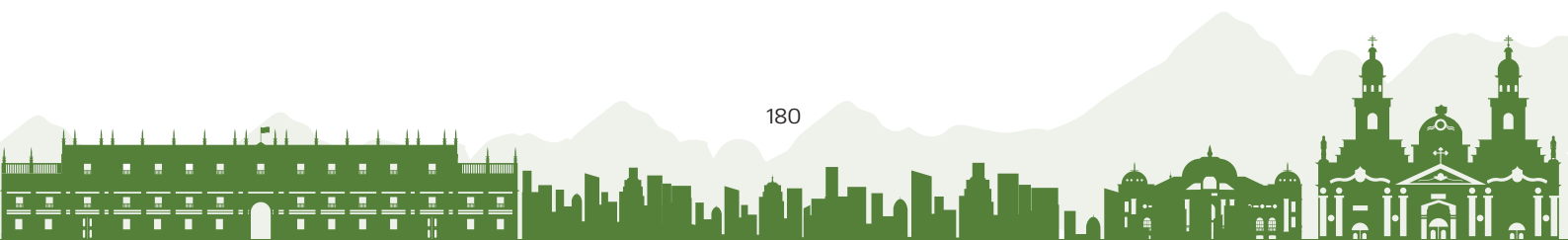
The most potent healing substances are present in the other eight main bee products, including propolis, beeswax, beehive air, royal jelly, Apilarnil, bee pollen, bee bread, and bee venom.

Learning how to collect, process, and use medicinal bee products is paramount not only for the health of beekeepers and their families but also for the local communities. They can benefit from the nutritional, preventative, and therapeutic properties of all beehive products.

Medicinal beekeeping is a higher form of beekeeping that emphasizes not only the cleanliness of bee products but also their richness in active pharmacological substances. These substances can prevent or heal hundreds of diseases, including infectious diseases that can be life-threatening, especially in isolated rural communities.

This presentation will discuss various medicinal beekeeping models, such as the one from New Zealand, which focuses on producing Manuka honey.

The presentation will also highlight the primary principles, rules, and legal regulations that ensure sustainability of medicinal beekeeping practices for rural beekeepers.





## Beekeeping for Rural Development

PP-116

### Generation of social capital between beekeepers and the University of O'Higgins

Andrea Julieta Müller Sepúlveda

Instituto de Ciencias Agroalimentarias, Animales y Ambientales, Universidad de O'Higgins

In Chile, beekeeping is mainly associated with Family Farming, where almost 80% of beekeepers carry out this type of production on a small scale. Due to the characteristics of these small beekeepers, it is vital that they can work together in order to have access to better benefits and prices for their products and services, but it has been seen that associativity is very low in this area. To support beekeepers in the O'Higgins region, a project entitled: "Generation of social capital between beekeepers and the University of O'Higgins, to improve the sanitary and epidemiological status of beehives in the O'Higgins region" was developed.

The objective of this project was to design and implement a trans-disciplinary linkage strategy between the beekeepers of the region and the University of O'Higgins, which would allow the construction of a "Road Map" to solve the existing gaps in the sector and define the lines of applied research to be developed. The aim is to improve the associativity of beekeepers, improve the health of the hives, pollination services and, therefore, regional beekeeping productivity.

To do this, the first thing we did was to gather information on the current situation of social capital together with the sanitary and epidemiological management of beekeepers in the O'Higgins region through a survey. Then, we determined the main problems and gaps associated with social capital, sanitary management practices and epidemiological status of regional bee production, through dialogue with different actors in the beekeeping sector by means of different workshops. And finally, we built a "Roadmap" with lines of research aimed at strengthening social capital to create value in production and that are relevant to improving bee health in the O'Higgins region.

This initiative is expected to improve integration, articulation and trust between the different actors in the beekeeping sector, as well as to focus future applied research. In addition, it will improve technology transfer, training and teaching in beekeeping production, bringing community members and young people in the region closer to the beekeeping sector.

PP-117

### Agricultural associations in the canary islands as instruments for the development of the regional bee fauna

M<sup>a</sup> Loreto Fuertes Díaz<sup>1</sup>, Esther Acosta Ballester<sup>1</sup>, Felisa Maria Hodgson Torres<sup>3</sup>, Loreto López De Vergara Fuertes<sup>4</sup>, Gladys Acosta Darias<sup>2</sup>

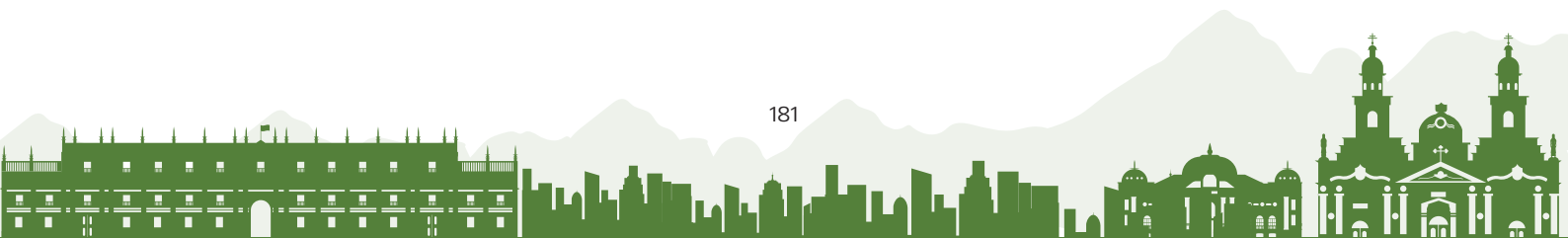
<sup>1</sup>Viceconsejería Sector Primario, Gobierno de Canarias, Santa Cruz de Tenerife, Spain

<sup>2</sup>Departamento de biodiversidad y conservación, Universidad de La Laguna, San Cristóbal de La Laguna, Spain

<sup>3</sup>Instituto de Calidad Agroalimentaria. Santa Cruz de Tenerife. Spain

<sup>4</sup>Asociación Ecored. Santa Cruz de Tenerife. Spain

Agricultural associations are a form of collective citizen participation in the primary sector of the Canary Islands. They are the bodies through which farmers and the population linked to the rural world can develop their functions of economic and social representation, as well as the collective activation of their businesses through training and dissemination of the values of the rural environment. The agricultural associative sector of the Canary Islands participates in a process of economic and social change, seeking the creation of wealth and its equitable distribution. Associations are an educating element for the individual, the collective and the valuation of the environment. They communicate, qualify and raise the cultural level of the professional collective of the rural world. Their participation and dynamisation is carried out with democratic practices. It deactivates individualism, promotes solidarity through cooperation, both of the rural and urban population. It contributes to raising the general economic level of the Canary Islands, improving efficiency and operational capacity; seeking to make the companies they group together profitable. They are an instrument for channelling actions to modernise the agricultural sector in the Canary Islands. They are promoted in the institutional framework emanating from the European Union and are developed by the territorial administrations of the States, in the Canary Islands through the regional and local governments. Due to the average size of farms in the Canary Islands, less than 2 ha, these associations bring together farmers from small local estates with a great diversity of agricultural and wild species that constitute a heritage to be preserved. An agricultural sector structured in the form of non-profit associations representative of their activity coexists alongside producer organisations in their different associative modalities. Most of the productive entities have achieved the status of regional Canary Islands Producers' Organisations. In both productive and non-productive organisations, the beekeeping sector, in recent years, has generated different types of productive development activities. We analyse the presence of beekeepers in these organisations, their role as instruments of cooperation with agricultural companies and the sustainable development of the rural environment.



## Beekeeping for Rural Development

PP-118

### The efforts of Saudi Arabia Government towards flourishing sustainable beekeeping

Ahmad Alkhazim Alghamdi

King Saud University

The efforts of Saudi Arabia Government towards flourishing sustainable beekeeping.

Dr. Ahmad Al Khazim Al Ghamdi

Bee Research chair, KSU, Saudi Arabia

Statistics and expert-estimations reported that around 14000 beekeepers managing more than one million colonies are established in Saudi Arabia. Honey production is estimated at round 2500 tons and honey import exceeds 24,000 tone of each year, annual package bee importations exceed one million pac ages.

The Ministry of Environment, Water and Agriculture is making remarkable efforts to develop and sustain the beekeeping industry of Saudi Arabia.

In 2019 and 2020 the Government launched several beekeeping development initiatives and projects, with an overall budget of exceeding one billion Saudi Riyals (350M USD); to be implemented within seven years starting from 2019.

These projects cover many aspects of the beekeeping industry and are mainly focused on:

- Selection and breeding of native honey bee ecotypes,
- Mass rearing of honeybee queens utilizing available queen rearing infrastructures managed by the ministry directorates. The main goal of the project is to produce about 50 thousand mated queens in three years period.
- Capacity building of local beekeepers and supporting start-up beekeeping materials for beginners. The project aims at training more the 2000 beekeepers (men and women) for 6 months (with a certificate of diploma) and supporting their start-up materials with 100 local honeybee colonies each?.
- Sustainable rural development project for rural communities that includes a beekeeping sector too targeting to boosts honey production, job creation and conservation of indigenous honeybee race through minimizing importation of exotic bees.

All projects will be implemented by the beekeepers' associations which are supported by the government with infrastructure and specialized experts.

Other stakeholders such as FAO (Food and Agriculture Organization), Agricultural Development Fund (ADF), Cooperative Associations Council (CAC).

PP-119

### Two Bees in a Podcast: Using Podcasts as an Effective Way to Communicate Apiculture Science

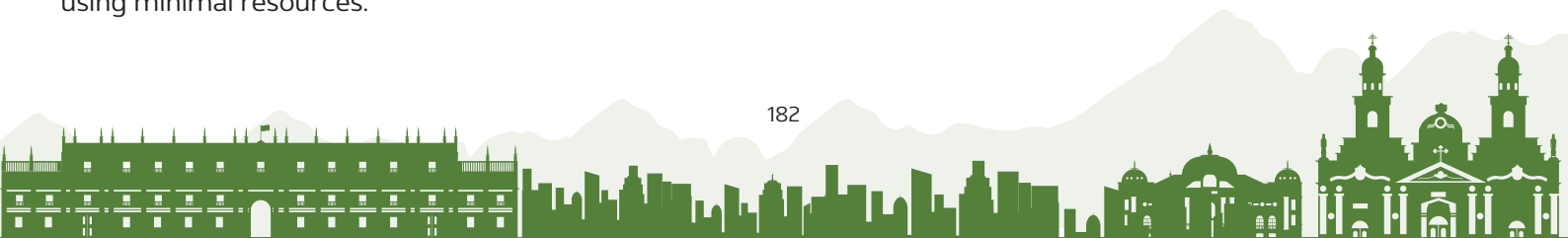
Amy Vu, James D Ellis

Department of Entomology & Nematology, University of Florida, Gainesville, Florida, USA

**BACKGROUND:** Agricultural communicators/educators must learn to develop new technology skills as mediums evolve. Podcasts are projected to become increasingly popular in the next decade, with audiences currently spending more time listening to podcasts than reading books, newspapers, magazines and using social media.

**OBJECTIVES:** "Two Bees in a Podcast" exposes listeners to honey bee research, highlights the beekeeping resources available at the University of Florida (USA) Honey Bee Research and Extension Laboratory (HBREL), and answers practical beekeeping questions from listeners. These questions typically focus on colony management, pests, disease, nutrition, pesticides, and other stressors. **METHODS:** U.S.-based and international honey bee researchers are invited to discuss their published research with the hosts from the HBREL. Hosts record two hours of audio each week. Episodes are recorded on Zoom and edited to create a full episode. Once complete, the episodes are uploaded to Anchor.fm and distributed on Apple Podcasts, Spotify, Anchor, Podbean, Google Podcast, Facebook, Twitter, an e-mail listserv, and the HBREL's website. Episodes are released weekly, with all episodes being 45 minutes to 1 hour in length. Listener data are collected and include the overall number of plays, weekly estimated audience and unique listeners, geographic location, gender, and age. Each episode has additional notes and resources available on the HBREL's website. **RESULTS:** Over 130 episodes have been released since 2020. The episodes have accumulated over 604,000 all-time plays, with an estimated audience of 2,609 listeners weekly. Listeners from >50 countries wait weekly for episodes to be released, complete an annual survey on use and behavior change, and provide feedback through e-mails.

**CONCLUSIONS:** Podcast production can be an easy way to create audio content as a method to disseminate honey bee resources internationally. They can be used to highlight international researchers and reach a global audience, while using minimal resources.



## Beekeeping for Rural Development

### PP-120

#### Space in beekeeping: no pink beekeeping overalls

Liane Oliveira Castilhos

Apiário Cambará

Beekeeping and the love of bees have always been part of my life. What I didn't imagine is that one day I would be a woman beekeeper and it would change my life to work with them. It all started well before, but in 2018 I came across my father, who took care of everything, sick and with honey to harvest from approximately 450 honeycomb. I didn't think, I just went. I believed that everything would work out and at my pace it is working. Only when I started to work effectively in our agroindustry I realized the dimension of what I had in my hands, even though t different ways my father had created and consolidated a brand. On the field it was a little more difficult. I faced situations of prejudice my rhythm that I had never experienced, on several occasions, I realized that I was acting as a helper for my helpers. It took a lot of determination and resilience to change this scenario. I managed, but I'm still fighting, because after all, structural machismo is rooted in the activity. We see many women with great jobs, but in the field day-to-day they are just supporting. We need to change this scenario (so that) more and more women take the course of their stories. When I realized this, I saw that the way would be long, after all, not even female Personal Protective Equipment (PPE) is available on the market, even though using the color pink does not necessarily mean that women's space is assured. Today I continue with a smaller number of honeycombs, I sell all our production directly to the end customer, and I seek to innovate and create new products with our honey every day. In partnerships, almost all of which are female, we strengthen ourselves so that together we can continue fighting, whether it be manufacturing candles or managing a honey warehouse. Discovering that they exist makes me happy, because even with the steps of little ants, together we are moving forward!

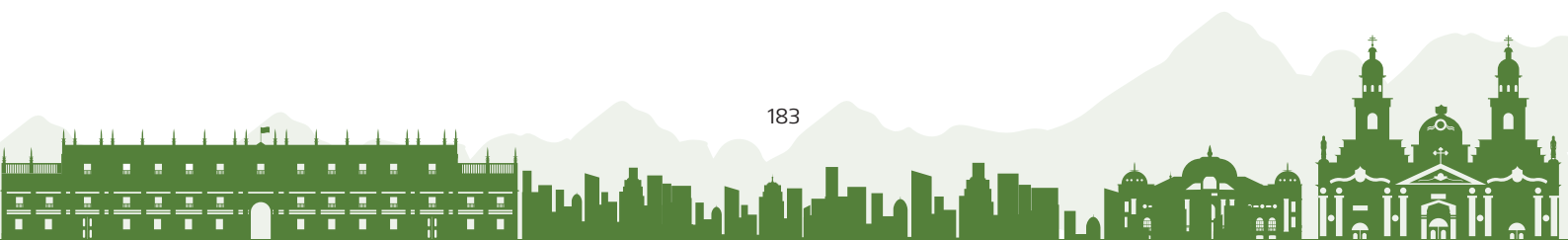
### PP-121

#### Beekeeping based on mutual welfare

Sonia Elizabeth Asimbaya Nasimba, Guido Ernesto Saldias Urzagaste

Chaco Bee Producers Association, Yacuiba, Bolivia

At present, having areas of natural forest to develop bee breeding is a luxury and is worrying at the same time, because the anthropic factor with "exploitation", is on our heels. In our conception of beekeeping, we work on the principles of animal welfare in the colony, being our GOAL: to provide welfare and comfort to bees in a natural forest, through holistic planning, recognizing that their retribution will be with production in harmony. The natural forest is located in the municipality of Yacuiba, Plurinational State of Bolivia, in the South American Gran Chaco, characterized by its tropical dry forest, with rainfall less than 600 mm. per year, high temperatures (> 40 ° C.), with tree species, shrubs and cacti that allow an important flowering period of 6 to 8 months (with the species *Ziziphus mistol* flowering for 3 months). It is essential to avoid stress nutritional, sanitary and hive management, for which we carry out the following resilient practices: monitoring of climate forecasts, rainfall records, maximum and minimum temperatures, flowering, permanent availability of clean water in the apiaries and the reading of the hives, which they allow us to replan activities, anticipating climate changes (ENSO Niño-Niña), where we went through the drought generated by La Niña for the third consecutive year. It is essential to consolidate a category 1 hive in May (dewormed), with 1.5 of a medium box with honey for its short wintering and through a precise management of stimulation, reach August 15 with 6 brood frames at the beginning of flowering. This has allowed us to achieve yields ranging from 45 to 66 kg/hive/year in the last five years. We conclude that it is necessary to reflect and act thinking about the welfare of the hives, recognizing their basic unitary needs and the colony, since many times beekeeping is done through practice and not articulated to comprehensive ethological knowledge. The things to improve, we will surely learn again along the way, with the most important Living Being on the Planet.



## Beekeeping for Rural Development

### PP-122

#### Strengthening of the beekeeping activity of the municipality of Delmiro Gouveia – AL through the Lagos do São Francisco Project

Renally Da Silva Medeiros<sup>1</sup>, Maria Amélia De Oliveira Silva<sup>1</sup>, Eva Sarmento Da Silva<sup>2</sup>, Rebert Correia<sup>3</sup>, Tania Maria Sarmento Silva<sup>4</sup>

<sup>1</sup>City Hall of Delmiro Gouveia, Alagoas, Brazil

<sup>2</sup>Collegiate of Zootechnics, Federal University of the São Francisco Valley, Petrolina, Brazil

<sup>3</sup>Brazilian Agricultural Research Corporation, Semirid, Petrolina, Brazil

<sup>4</sup>Departament of Chemistry Federal Rural University of Pernambuco, Recife, Brazil

The present work aimed to present the transformations that the Project "Development actions for students and agricultural producers – Beekeeping Action Plan for the region of the Lakes of São Francisco – PE/SE/AL/BA" provided to its beneficiaries in the municipality of Delmiro Gouveia – AL. The research was conducted through semi-structured interviews with a previous script, applied in a direct and interactive way, in loco, with the beekeepers participating in the Action Plan in the municipality, configuring itself as an action research of qualitative and quantitative nature. As a result of the actions developed by the beekeeping plan, it was found that of the 10 beekeepers participating in the Project, 08 (80%) remain in the activity, with a total of 201 boxes, of which 145 are populated (72.1%), culminating in a total production of 1,623 kg of honey per harvest and average production of  $202.8 \pm 106$  (mean  $\pm$  standard deviation) kg per producer.

The production is marketed directly to the consumer, in free and/or door-to-door fairs, in which 75% of the beekeepers benefiting from the Project have the activity as secondary income. The Action Plan provided significant changes in the beekeeping activity of the municipality, through educational, social, financial, economic and environmental technical practices, strengthening and structuring beekeeping on a sustainable basis in family properties.

### PP-123

#### Organizational development plan as a value proposition of the cooperative of beekeeping producers Copap Ltda

Victor Alberto Rusas, Javier Roberto Vagabculov, Roberto Alonso Saez, Daniel Hugo Codutti

Instituto Nacional de tecnologia agropecuaria Colonia Benitez Chaco Argentina

The beekeeping cooperative Copap Ltda. from Chaco Province, founded in 2005 by 14 members, promotes the production and commercialization of honey, pollen, propolis, wax, and extraction and packing services to local producers.

The organization did not have its business mission and vision. Likewise, a series of factors limited its development: low young people and women participation, low productive scale, low average yields, incipient value-added processes, unregistered trademark, informal marketing channel, lack of planning tools and zero horizontal integration.

Based on an improvement in management, it was proposed to work under the objective of "designing and applying a participatory plan for sustainable development".

Applying the "collective way of innovating" method, the results were obtained in the following dimensions:

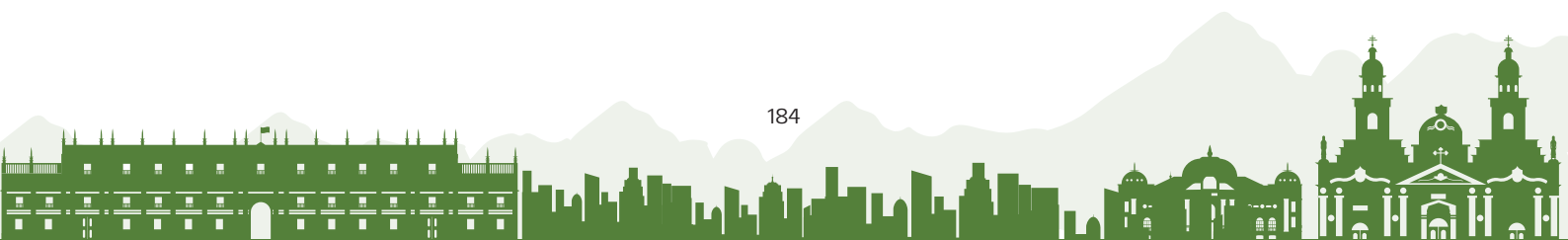
Social: gender inclusion in activities, incorporating 12 new members (4 female) and the training of 47 new beekeepers (29 young people and 18 women); a technological linkage agreement with INTA - EEA Colonia Benítez, Cambio Rural Program and INTA PROAPI (Programa Nacional Apícola); the Organizational Mission and Vision were defined, and the design and implementation of a sustainable development plan.

Commercial: application of HACCP standard, gluten free, obtaining collective worldwide trademark registration; different packaging (blisters, sachets, glass containers, and PVC); 14 new commercial channels in Argentina; organic certification of apiaries, incorporation of QR code in fractionated honey; and commercial integration with Chaco Marketing Consortium.

Productive: 80% increase in average yield per hive; and 400% increase in the number of honey drums.

Environmental: design and application of an environmental planning tool, with criteria for productive technical management of apiaries based on the flowering curve in each environment.

It is concluded that this work contributed to organizational improvement, beekeepers' life quality and environment protection.



## Beekeeping for Rural Development

### PP-124

### Redesigning beehives to be affordable, efficient in pest management and having the combined advantages of standard bee hives-the case of 'intelligent' beehive hive concept

**Abraham Addo Ansah Allotey**

Allotey Honey Bee Farms (Allotey Apiaries)

#### Objectives;

To produce an appropriate beehive that sustainably uses low cost constructional materials (as in traditional hives, both wood & non wood) but with the efficiency of the transitional and modern hives coupled with being able to manage bee pest sustainably

#### Introduction;

Africa is endowed with various various beehive construction materials that are being used for traditional beehive construction however those traditional hives are not very productive in terms of honey production due to their shapes (designs) and sizes. Also the prevailing pest incidence of Varroa mites, Small Hive Beetle Wax Moth infestations make the re-use of honey extracted comb to increase honey yield less desirable in Africa and also amongst top bar hive users The 'Intelligent beehives' principles is therefore being promoted to enable the use of 'green', low-cost or virtually free beehive constructional materials and also to reduce hive pest infestation through modification of hive entry holes and the establishment of pest traps along both the hives' internal and external bodies

#### Materials and Methods;

The box-like framework could be made of plastic, metal and wood with three hive cover slot-able sections on all the hive's sides which are further subdivided into either two (2) or four (4) open spaces except the base covers which has only two (2) openings

The design enables wood or non-wood to be used to covers the open spaces.

#### Results

The intelligent hive has the following benefits

To produce more green low cost efficient hives

It has an in-built pests traps both external and internal of the hive

The hive bodies could be removed and sanitized whilst the hive is in session

It has bottom ventilation system

#### Discussions and Conclusions

The framework hives is therefore a platform on which any material can be fixed on for hive construction. It has a knock-down fixture and the cover materials could be replaced whilst in service. The framework hives enable all standard hives to be fabricated including cylindrical hives. The metal framework hives with floor tiles or burnt clay slaps as covers could be used in prone areas to protect the beekeeper's investment

### PP-125

### Advance engineered top bar is africa's answer to the langstroth frames- for improved honey harvesting of over 100% yield without comb destruction in top bar hives

**Abraham Addo Ansah Allotey, Anna Adukwei Allotey**

Allotey Honey Bee Farms (Allotey Apiaries)

#### Objective;

To increase honey production in both quality and quantity to over 100% within the African Honey Industry

Introduction; Africa is importing three times the honey she produces even though she has healthy, industrious and large population of bees in addition to variable vegetation. This is due to the mechanism of honey harvesting that destroys the combs and thereby reducing rate of harvesting and honey production.

The Advance Engineered Top Bar (AETB) provides that answer. It can be made of wood, plastic, metal and their combinations that can be fabricated (except the plastic type) by tin / metal smith, welders and carpenters at the village level without any very complex machines.

Foundation combs are placed within the AETB for the bees to form their combs over its mesh to prevent comb breakage during comb movement and honey harvesting. Without foundation combs the bees will work similarly as above.

#### Material and Methods

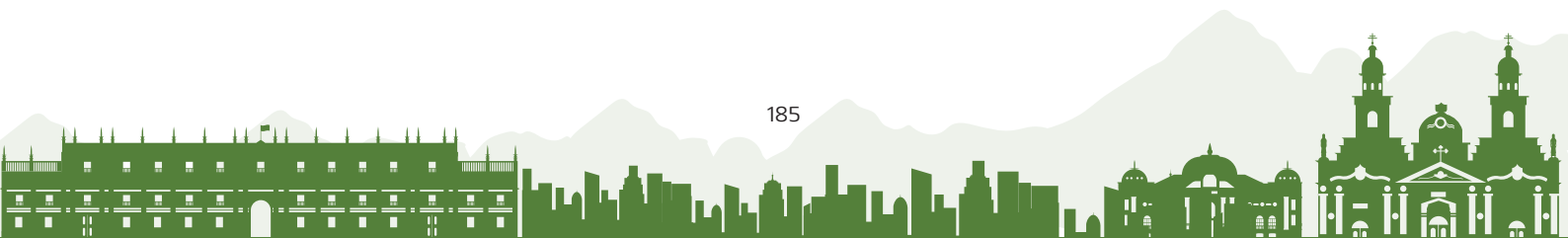
It can be made of wood, plastic and metal. The simplified version is a square frame of dimension 38\*38 cm with one of the sides extended to a length of 48cm to act as handles. The top bar and the bottom bars are held together in place by vertical rods of length 20-22 cm which can be adjustably positioned according to the width of the hive to fit into any type of hive

#### Results

The AETB enables the bees to form their combs over its mesh to prevent comb breakage during honey harvesting and hive movement during provision of pollination services. Similarly honey laden combs on top bars will produce natural foundation combs when also placed in the AETB for honey extraction in centrifuge extractors as exhibited by Langstroth Frames.

#### Discussions and Conclusions

The AETB can fit into any hive type as against the Langstroth frames that fit into only its hive types. The AETB has a moving rod system that is adjustable depending on the width of the hive for which the bees form their combs over its mesh to prevent comb breakage during honey harvesting.





## Beekeeping for Rural Development

### PP-126

#### Apiculture: increasing the income of small beekeepers in the rural area of Sertão do São Francisco-Bahia

Eva Monica Sarmiento Da Silva<sup>1</sup>, Rebert Coelho Correia<sup>2</sup>, Tania Maria Sarmiento Da Silva<sup>3</sup>, Renally Da Silva Medeiros<sup>4</sup>, Eljalma Augusto Beserra<sup>5</sup>

<sup>1</sup>Collegiate of Zootechnics, Federal University of the São Francisco Valley, Petrolina, Brazil.

<sup>2</sup>Brazilian Agricultural Research Corporation, Semirid, Petrolina, Brazil

<sup>3</sup>Departament of Chemistry Federal Rural University of Pernambuco, Recife, Brazil

<sup>4</sup>City Hall of Delmiro Gouveia, Alagoas, Brazil

<sup>5</sup>Collegiate of Post-Graduation in Agroecology and Territorial Development, Federal University of the São Francisco Valley, Petrolina, Brazil.

The present work aimed to turn the breeding of ("Apis mellifera") bees into a profitable activity for small farmers in dry areas around wind farms in the municipality of Casa Nova, BA. The research was carried out in the municipality of Casa Nova, BA with small family producers. The selection was made through interviews using semi-structured questionnaires, using as a criterion the affinity with the beekeeping activity. In total 18 participants were selected, being 14 male and 04 female, they did not have experience in the apicultural activity, and they worked in the areas of cultivated crops. Beekeeping emerged around the wind farm areas from a problem identified during the dry season, since the honey bees migrated from the rain dependent area to the surroundings of the wind farms, due to the availability of water and food. Being a region with many fruit trees, mainly mango and guava, it was verified that the producers/entrepreneurs, when they found swarms in the fruit trees, eliminated them by using fire or other means so that the workers could perform their activities in the fruit trees, without the risk of being attacked. To start the rational breeding the beekeepers received all the necessary material for rational breeding, as well as were capacitated from extension activities, in which they got a satisfactory production in average 30/kg/box/year. Moreover, the beekeeping flora of the region favors the activity in the municipality, as well as the increase in the beekeepers' income, besides the ecological benefits, due to the pollination of the fruit trees by Africanized bees.

Acknowledgment: CNPq, CAPES, FACEPE

### PP-127

#### Apicultural craftsmanship and the settlement of young people in the semiarid region of pernambuco

Eva Monica Sarmiento Da Silva<sup>1</sup>, Eljalma Augusto Beserra<sup>2</sup>, Maria Helena Maia e Souza<sup>3</sup>, Maria Augusta Maia e Souza Beserra<sup>4</sup>, Tania Maria Sarmiento Da Silva<sup>5</sup>, Yan Lima<sup>6</sup>

<sup>1</sup>Collegiate of Zootechnics, Federal University of the São Francisco Valley, Petrolina, Brazil.

<sup>2</sup>Collegiate of Post-Graduation in Agroecology and Territorial Development, Federal University of the São Francisco Valley, Petrolina, Brazil.

<sup>3</sup>Collegiate of Psychology, Federal University of the São Francisco Valley, Petrolina, Brazil.

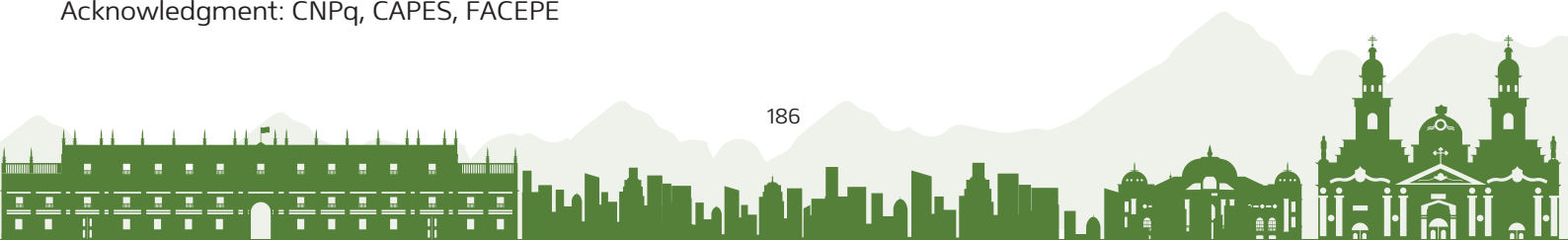
<sup>4</sup>Collegiate of Medicine, Federal University of the São Francisco Valley, Petrolina, Brazil.

<sup>5</sup>Departament of Chemistry Federal Rural University of Pernambuco, Recife, Brazil

<sup>6</sup>Paulista State University Julio de Mesquita Filho, São Paulo, Brasil

The mechanization of the field has mitigated the labor force used in agriculture, leading young farmers to seek the urban areas of the cities, in search of education, work and housing, potentiating the social issues of periurban regions. The lack of jobs for young peasants was the reason for the search of irrigated areas in the São Francisco Valley, compromising the food sovereignty of family farmers. The Companhia de Desenvolvimento dos Vales do São Francisco e Parnaíba - CODEVASF, through the Tomorrow Project, has promoted the socioeconomic inclusion of young farmers, between 14 and 26 years old, in the labor market, in this perspective, in the period from 2019 to 2022, 223 rural young people, in 16 municipalities in the state of Pernambuco, participated in training in beekeeping (108), processing of apiculture wax (42) and production of honey-based cosmetics (73). Of the courses offered, those that showed the greatest potential for professional inclusion were those related to beekeeping, especially the production of cosmetics. This finding is directly related to the potential of beekeeping developed in the semiarid region of Pernambuco, as well as reflects the process of urbanization of rural areas, where products linked to the preservation of the environment began to be sought by a niche population that values activities that enhance socio-environmental elements. Focused on this market, the courses honored the cultural and environmental characteristics of the regions where they were applied, using local knowledge and materials available in the region, seeking to reduce waste, adding economic value to inputs that would be discarded or even underutilized, as a result of this processing, the products of family beekeeping, has promoted increased income, and contributing to the socioeconomic freedom of young beekeepers attended.

Acknowledgment: CNPq, CAPES, FACEPE



## Beekeeping for Rural Development

PP-128

### Beyond Aid. Organized Beekeeping for Rural Social Economic Transformation: A Case Study of Happy Community Organization Beekeeping Project in Kenya

Linnah Koyiano Resuka

Department of Management Science & Entrepreneurship - Moi University

For over 50 years, investments have been made to develop the beekeeping value chain in Kenya. Multisectoral stakeholders agree that the sector has potential to among others transform rural households' economies. However, the sector remains stagnated. Initiatives have mainly focused on tools and equipment donations to rural household. There are few successes from such models which are especially popular with both government and non-governmental organization. Two critical truths are missed in the popular method. The first truth is that beekeeping is both a science and an art. The second truth is that it is local. These two accepted truths in the beekeeping world are critical in shaping how a successful model is to be created. The current models in place fails to appreciate the role of this truths. At the heart of the truth is appreciating bee biology, behavior, beekeeping seasons and the environment. A successful beekeeper is informed by cultivating such truth. The beekeeping practice of such a beekeeper is heavily influenced by a natural introspection of the bee in the wild. Therefore, building successful beekeepers goes beyond donations of tools and equipment. It is beyond a one man show and in Africa. It seeks to develop the beekeeper's knowledge of such truths. it is therefore a communal responsibility that should be at the foundation of efforts to develop beekeeping in rural economies.

PP-129

### The notoriety of the Serra da Capivara Territory, Caatinga dry forest, in honey production

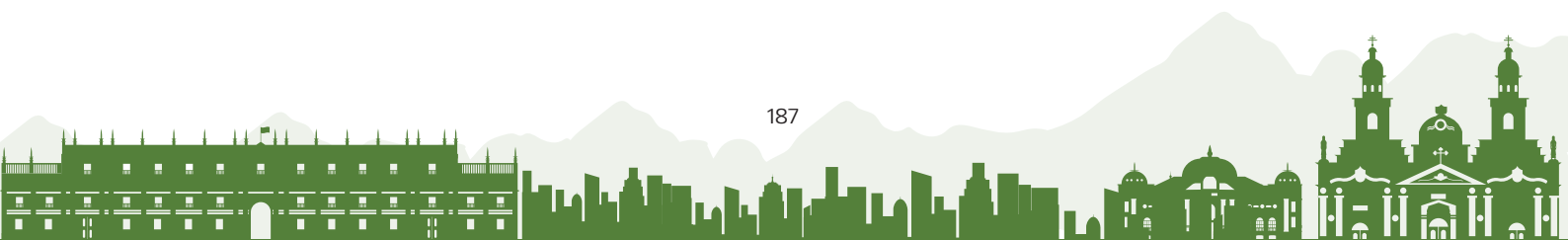
Liária Nunes Silva<sup>1</sup>, Juliana Nascimento Bendini<sup>2</sup>, Darcet Costa Souza<sup>2</sup>, Sinevaldo Gonçalves Moura<sup>2</sup>, Lenita Delmondes Cardoso<sup>1</sup>, Patricia Maria Drumond<sup>3</sup>, Salatiel Amorim Barbosa<sup>1</sup>

<sup>1</sup>Federal Institute of Education, Science and Technology of Piauí, São João do Piauí, Brazil

<sup>2</sup>Federal University of Piauí, Brazil

<sup>3</sup>Brazilian Agricultural Research Corporation, Teresina, Brazil

The Serra da Capivara Territory is situated in Caatinga dry forest, state of Piauí, Brazil. The region concentrates the high biological richness of the Caatinga Biome, in addition to the largest number of archaeological sites with cave paintings in the world, being a Cultural Heritage of Humanity. The region is also recognized for the occurrence of Agrarian Reform projects and quilombolas communities. Among its economic and productive potential, beekeeping stands out, an activity that has been strengthened over time, based on collaborative work between beekeepers and public and private institutions. This research presents, through a notoriety dossier, the history of beekeeping practiced in the Serra da Capivara territory, highlighting historical milestones and the notoriety of the territory in the national scenario as a honey extraction and production center. The data presented show the Territory's notoriety as a center for the production and export of organic honey, a fundamental requirement in requests for an Indication of Origin (IP), whose establishment has the potential to contribute to the strengthening of beekeeping in the demarcated area and may represent economic impacts, social, environmental and technological. It was observed that one of the main differentials of honey from the Serra da Capivara Territory, in addition to its color and aroma, is its organic production, since no pesticides are used in the small plantations that are within the production territory, maintaining the natural characteristics of the flowering of the Caatinga Biome. The knowledge of small rural producers, many from traditional communities, characterized by the establishment of a harmonious relationship with this exclusive and biodiverse ecosystem, results in a unique product whose peculiarities may be able to bring competitive advantage, market differential and reputation to the Territory.



## Beekeeping for Rural Development

PP-130

### Scientific divulgation concerning to bees and beekeepers. The case of EUNK Magazine

María Emilia Estrada<sup>1</sup>, Eliana Gabriela Revello<sup>2</sup>, Giuliano Maximiliano Cernada<sup>2</sup>

<sup>1</sup>Departamento de Economía, Universidad Nacional del Sur; Laboratorio de Estudios Apícolas (LabEA - UNS)

<sup>2</sup>Cooperativa de Trabajo Apícola Pampero Ltda.

Scientific journals are traditionally the main channel for scientific communication, quality control of research results and preservation of scientific advances, in a broad sense (both pure and natural sciences and social sciences). In addition, scientific dissemination and diffusion are relevant processes for promoting innovation and achieving academic and social impact. On the other hand, the importance of academic journals in Latin America, which currently lag behind in scientific production, is recognized. This paper investigates the role of different public and private institutions and Non-Governmental Organizations (NGOs) in the dissemination of scientific research related to the function and problems of bees and beekeepers through an exhaustive national and international bibliographic review. As a particular case, the case of the EUNK Journal is analyzed, created in the year 2021, from the joint work of four beekeeping research centers belonging to: Universidad Nacional del Sur (UNS), Universidad Nacional de Mar del Plata (UNMdP), Universidad Nacional de La Plata (UNLP) and Universidad Nacional del Nordeste (UNNE), and two associative strategies: the Cámara de Apicultores Pampero (CAP) and the Cooperativa de Trabajo Apícola Pampero Limitada (CTAPL). This case is of interest because it proposes: i) an integral vision presenting studies on bees, beekeeping and beekeepers at national and international level. It seeks to bring together contributions from the natural and social sciences and generate synergies. ii) A strategic and long-term vision, an aspect summarized in the name of the journal, which means "to look far". iii) Strengthen research advances in Latin America, particularly in Argentina, seeking to accompany and promote the outstanding historical performance of beekeeping in terms of production, pollination, exports, cooperativism and innovation.

PP-131

### Socioeconomic profile of female beekeepers and meliponiculturists in the north of the state of bahia

Catiana Da Conceição Veira Melquiades<sup>1</sup>, Yan Souza Lima<sup>2</sup>, Francisco Israel Lopes Sousa<sup>1</sup>, Lucas Moura Ribeiro Afonso<sup>1</sup>, João Henrique Alves Da Cruz Coelho<sup>1</sup>, Juliana Duarte De Brito Campos<sup>1</sup>, Eljalma Augusto Bezerra<sup>1</sup>, Eva Monica Sarmiento Silva<sup>1</sup>, Tania Maria Sarmiento Silva<sup>3</sup>, Gerlania Sarmiento Verissimo<sup>4</sup>, Rebert Coelho Correia<sup>5</sup>

<sup>1</sup>Department of Zootecnics, Federal University of São Francisco Valley, Petrolina, Pernambuco, Brazil

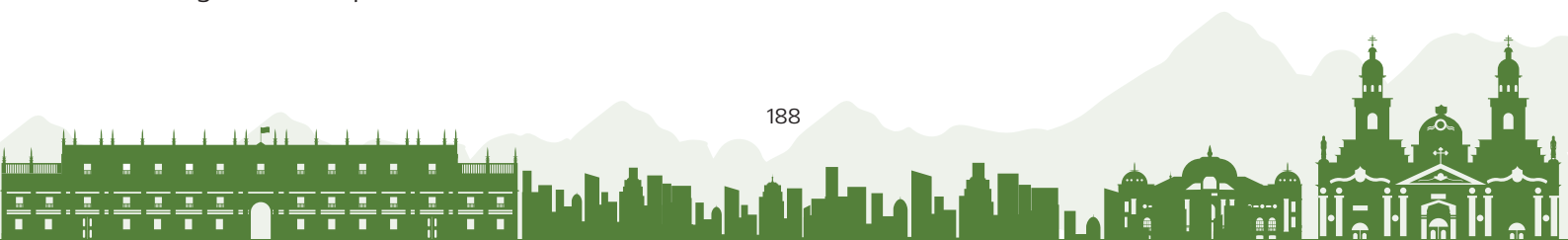
<sup>2</sup>Paulista State University Julio de Mesquita Filho, Botucatu, São Paulo, Brazil

<sup>3</sup>Department of Chemistry Federal Rural, University of Pernambuco, Recife, Brazil

<sup>4</sup>University Hospital Lauro Wanderley, João Pessoa, Paraíba, Brazil

<sup>5</sup>Brazilian Agricultural Research Company for the Semiarid, Petrolina, Pernambuco, Brazil

The objective of this research was to analyze the contributions of the MeliApis project in the socioeconomic development of women who live in the municipalities around Lago de Sobradinho (BA.). The project MeliApis had the proposal to insert and empower women in the sustainable management of native and honeybee breeding, since beekeeping and meliponiculture. The research was carried out in five municipalities located on the banks of the Sobradinho Lake (Bahia State): Casa Nova, Pilão Arcado, Remanso, Sento Sé and Sobradinho, with the objective of characterizing the socioeconomic and productive profile of the women who participated in the project. The data were obtained by applying a semi-structured questionnaire and the participation of 24 women. Before the application of the actions promoted by the project, beekeeping and meliponiculture were practiced by 33% and 13% of women, respectively, and both activities were performed by only 4%. After the MeliApis project, all participants became beekeepers and 58.3% beekeepers of stingless bees. The main product obtained is honey from *Apis mellifera* (75%), which had an increase in production of 457% after the project. Before the production was 1,126 kg/year and jumped to an average production of 6,284 kg/year. The beekeepers' income due to honey production increased 72.2%. Through beekeeping and meliponiculture, the women who were contemplated with the project showed potential for income generation and high impact on the development and improvement of lives in the countryside. Acknowledgment: CNPq, CAPES and FACEPE.



## Beekeeping for Rural Development

### PP-132 [Beekeeping for Rural Development]

#### Exploring socioecological systems in apiculture: A study of *family farming communities* in the 8<sup>th</sup> region of Chile

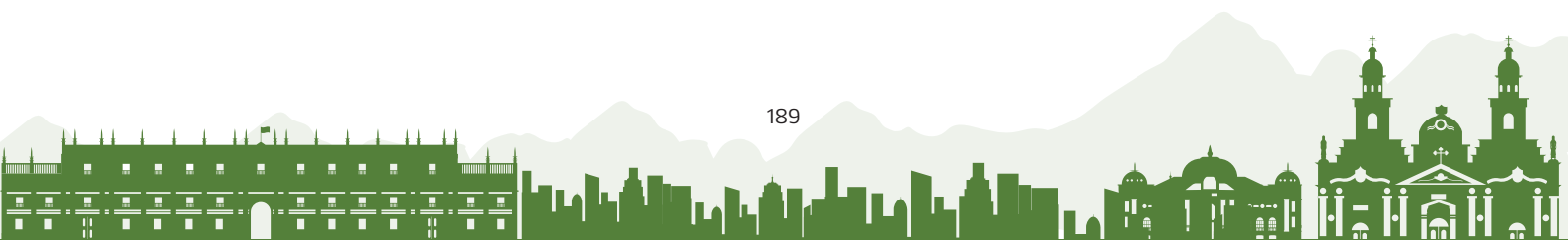
Martín García

Departamento de Sociología, Universidad de Concepción, Concepción, Chile

This study focuses on the socio-ecological systems of artisanal beekeeping in rural communities in the provinces of Biobío and Concepción in the VIII region of Chile. The research aims to characterize the population of beekeepers in the area and identify the variables that directly affect the quality of honey. The study also aimed to characterize the socio-ecological and historical spatial context in which small and medium-sized beekeepers operate. The data were collected through a quantitative survey that included variables such as age, gender, educational level, type of assistance during the harvest, belonging to social groups, belonging to INDAP, the degree of dedication to beekeeping, and knowledge of beekeeping, among others.

The results of the study provide information on the characteristics of small-scale beekeeping in the region and suggest that factors such as educational level and membership in social groups could be associated with a higher quality of honey production. In addition, the study reveals the importance of considering the historical and socio-ecological context of the communities in which beekeeping takes place in order to understand the dynamics of small-scale beekeeping.

The study's findings may be useful to policy makers and stakeholders in supporting small-scale beekeeping in the region. The results also highlight the need for more research to explore the complexities of socioecological systems and the role of historical and cultural contexts in shaping them. Overall, the study contributes to the growing body of literature on socioecological systems and the importance of incorporating social and ecological factors in understanding environmental problems.



### PP-133

#### Pollinifer flora associated with cliserie forest systems in productive apiculture systems in Boyacá, Colombia

Guillermo Salamanca Grosso, Monica Patricia Osorio, Fredy Alexander Borja Peralta

Mellitopalynological Research Group, Department of Chemistry, Faculty of Sciences. University of Tolima

Bee flora is the set of plant species, wild or cultivated, capable of attracting bees, which obtain their nectar, pollen, and other valuable resources for the hive. Floristic surveys play a distinct role when it comes to the establishment of plant-insect relationships. In the case of beekeeping, these species allow us to determine the botanical origin of honey, pollen, and other products. In this study, the flora of relevant apicultural interest in the cliserie forest in the municipalities of Belén and Cerinza (Boyacá), which is related, comprises 106 botanical species, distributed in 42 families in which the Asteraceae group predominated in some species with 27% of the total, followed by Ericaceae with 12% and Melastomataceae with 7%. In relation to the predominant specimens found were Asteraceae (*Baccharis* sp, *Taraxacum officinale*, *Bidens* sp.), Cunnoniaceae (*Weinmannia tomentosa*), Melastomataceae (*Miconia* sp., *Monochaetum* sp.), Sapindaceae (*Dodonea viscosa*), Fabaceae (*Trifolium repens*), Myricaceae (*Morella parvifolia*), Elaeocarpaceae (*Vallea estipularis*), Myrtaceae (*Myrcianthes leucoxylla*), Verbenaceae (*Duranta mutisii*), Ericaceae (*Bejaria resinosa*, *Macleania rupestris*, *Gaultheria* sp, *Pernettya* sp) mainly. In addition, a pollen atlas and a description of the main pollen types existed. A technical taxonomic and phenological document was structured to contribute to the economic and sustainable management of beekeeping, encouraging environmental conservation and responding to the knowledge of the dynamics of the plant species that are exploited from the benefits systems of beehives in the different Colombian biogeographic zones.

### PP-134

#### Pollination biology of the melittophilous species *Calophyllum brasiliense* Cambess. (*Calophyllaceae*) in a fragment of dense rainforest in São Paulo (Brazil)

Natalia Sênedá Martarello<sup>1</sup>, Adriana De Oliveira Fidalgo<sup>2</sup>, Favízia Freitas De Oliveira<sup>3</sup>, Mônica Lanzoni Rossi<sup>4</sup>, Adriana Pinheiro Martinelli<sup>4</sup>, Eduardo Luis Martins Catharino<sup>5</sup>, Cynthia Fernandes Pinto Da Luz<sup>1</sup>

<sup>1</sup>Laboratório de Palinologia PALINO-IPA, Instituto de Pesquisas Ambientais, São Paulo, Brazil

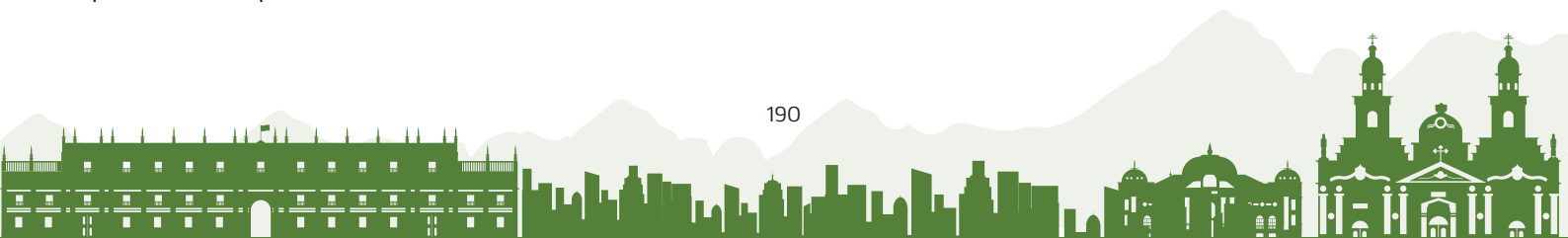
<sup>2</sup>Laboratório de Pesquisa em Sementes, Instituto de Pesquisas Ambientais, São Paulo, Brazil

<sup>3</sup>Laboratório de Bionomia, Biogeografia e Sistemática de Insetos, Universidade Federal da Bahia, Salvador, Brazil

<sup>4</sup>Laboratório de Biotecnologia Vegetal, Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, Brazil

<sup>5</sup>Herbário SP, Instituto de Pesquisas Ambientais, São Paulo, Brazil

*Calophyllum brasiliense* is a melittophilous tree with a neotropical distribution. Studies on its reproductive biology are limited and incomplete. The objective of this work was to describe the reproductive biology of *C. brasiliense* in the Parque Estadual das Fontes do Ipiranga in São Paulo (23°38'21.6"S 46°37'41.3"W), evaluating the reproductive system through controlled pollinations. Pollen viability was determined by 2,3,5-triphenyl tetrazolium chloride (TTC, 5%) and stigmatic receptivity was evaluated in hermaphroditic individuals using hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub>, 3%). Floral visitors were observed, collected, identified and deposited in the collection of the Museu de História Natural of Bahia (MHNBA). Pollen analysis was carried out under optical, scanning and transmission electron microscopy in perfect flowers from hermaphroditic individuals, and staminate and perfect flowers from andromonoecious individuals, applying multivariate statistics. The cross-pollination between perfect flowers of andromonoecious and hermaphroditic individuals presented 8.8% of success; cross-pollination between staminate flowers and complete flowers of hermaphroditic individuals 7.7%, and the control group 8%. In andromonoecious individuals, hermaphroditic flowers showed pollen viability of 66.38% and staminate flowers 55.78%. The perfect flowers of the hermaphroditic individuals showed 99.94% of non-viable pollen grains and receptive stigma throughout the entire anthesis. The main potential pollinators in *C. brasiliense* were *Apis mellifera* (8.61%), *Paratrigona subnuda* (5.08%) and *Tetragonisca angustula* (42.38%). *Apis mellifera* was considered a 'pollen robber' when it forced the opening of flower buds and *T. angustula* a 'secondary robber' when taking advantage of perforations caused by wasps in pre-anthesis buds to access it. *Plebeia droryana*, *Augochloropsis* sp.1 and *Trigonisca (Leurotrigona) muelleri* were characterized as 'pollen thieves' only when visiting andromonoecious or hermaphroditic individuals and not promoting pollen transport between them. *Brachygastra lecheguana* and *Vespidae* sp. 1 showed a high frequency of visits but were predators of larvae that parasitized flower buds. The pollen grains of *C. brasiliense* are monads, isopolar, medium sized, oblate-spheroidal to prolate-spheroidal, 3-(4)-zonocolporate, with microreticulate-microequinate sexine ornamentation, with the morphotype of hermaphroditic individuals presenting the smallest grains and the largest percentage of abnormal pollen. It is concluded that this plant species has natural low reproductive success and dependence on pollinators for fruit and seed set.





## Pollination and Bee Flora

### PP-135

#### Production of melon (*Cucumis melo*) under a greenhouse using the bumblebee native *Bombus pauloensis* and honeybee as pollinators

Sheena Marie Salvarrey<sup>1</sup>, Fernanda Zaccari<sup>2</sup>, Yulai Ramos<sup>1</sup>, Luis Barboza<sup>1</sup>, Leandro Arrillaga<sup>2</sup>, Olga Pascual<sup>2</sup>, Diego Romero<sup>2</sup>, Ciro Invernizzi<sup>1</sup>

<sup>1</sup>Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

<sup>2</sup>Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay.

In Uruguay the production of melon (*Cucumis melo*) under greenhouse out of season It has had a sustained growth favored by high prices. this crop has a high dependence on entomophilous pollination (70%), which is why it is usually used to pollination by honey bees, despite the difficulties they have to work in greenhouses. The use of bumblebees, recognized for its good performance inside the greenhouses, could contribute in a more efficient way in the fruit production. This work is aimed at evaluating the performance of bees honeydew and the native bumblebee *Bombus pauloensis* as pollinators of low melon greenhouse. For this, four colonies of bumblebees were installed in a melon crop under a greenhouse of 800 m<sup>2</sup>, while honey bees came from hives nearby. Insect activity occurred within the greenhouse and they were marked flowers visited by honey bees and bumblebees. Once the fruits are formed from the marked lowers these were harvested and processed in the postharvest laboratory of the Faculty of Agronomy, where different performance indicators. The results showed that the fruits obtained from flowers visited by bumblebees at greater weight per fruit, diameter, diameter of seminal capacity, weight of 100 seeds and shell thickness than those obtained by flowers visited by honey bees ( $P < 0.05$ ). Regarding the activity of insects, it is verified that the presence of honey bees will arrive after the installation of the bumblebee nests, which could be explained by competitive exclusion. This studio shows that the use of bumblebees as melon pollinators in greenhouses can improve yield and quality of fruits.

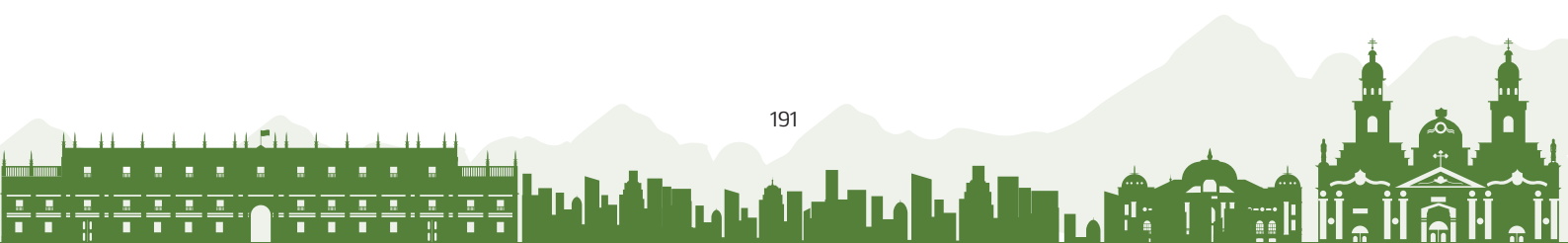
### PP-136

#### Value of National Forest as Honey Resources and Pollinating Insects in Korea

Seunghwan Lee

Depart of Agricultural Biotechnology, Seoul National University, South Korea

Providing continuous nectar sources for honey bees is essential for the beekeeping industry. However, we are experiencing the shortage of honey plants due to the increase of industrial and/or agricultural use of land, not only in the urban area but also in the rural region. It becomes even worse because the flowering plant are effected by the climate change and the other environmental factors, e.g. plant pathogens and insect pests. In this context, the national forests may serve major honey resources for the beekeeping industry and the habitats for the wild pollinating bee species. As the model, two national forests are surveyed and evaluated as the value of resource for honey plants and wild bee diversity in the central region of the Korean Peninsula. The native honey plants are surveyed for their blooming season and the foraging bee diversity is also investigated. For the main honey trees, the honeybee preferences are calculated by counting all the foraging bees, including the native wild bee species.



## Pollination and Bee Flora

### PP-137

#### Non-apis Bees As Pollinators Of Brazil Nut (*Bertholletia Excelsa*, *Lecythidaceae*) Flowers In The Brazilian Amazon And Pollinator Friendly Farming Practices

Márcia Motta Maués<sup>1</sup>, Marcelo Casimiro Cavalcante<sup>2</sup>

<sup>1</sup>Laboratório de Entomologia, Embrapa Amazônia Oriental, Belém, Brazil

<sup>2</sup>Instituto de Desenvolvimento Rural, Universidade da Integração Internacional da Lusofonia Afro-Brasileira, Redenção, Brazil

The magnificent Brazil nut (*Bertholletia excelsa*, *Lecythidaceae*) is native tree distributed in the forests of Brazil, Guianas, Colombia, Venezuela, Peru, Bolivia and Brazil. It produces hard globose fruits bearing 10 to 25 “nuts” which are essentially seeds, rather than nuts, but popular designation prevails. These seeds sustain one of the most important extractive industries in Neotropical forests, where nuts are harvested in natural forests and internationally traded over 130 years, generating income fostering bioeconomy. The Brazil nut is a key Non-Timber Forest Product (NTFP) component in Extractive Reserves in the Brazilian Amazon and has also been cultivated in agroforestry systems and monoculture with high pollinator’s diversity. The main flowering period extends from October to January, with large inflorescences bearing yellow attractive flowers visited by almost 30 species of medium to large native bees, strong enough to open the peculiar flower structure to collect pollen and nectar, a required condition to pollinate the flowers, as the plant is cross-pollinated, and totally dependent on biotic pollinators. Non-*Apis* native bees of the genus *Bombus*, *Centris*, *Xylocopa*, *Eulaema* and *Epicharis*, native and most of them solitary bees, are the effective pollinators in natural and cultivated areas of Brazilian Amazon. Pollinators surveys in agricultural systems with different landscape surroundings has shown that orchards close or surrounded by native forest present higher pollinators’ species richness when compared to areas where most of the landscape was converted to agriculture. In order to enhance population of Brazil nut pollinators, best management practices should preserve the natural forests or secondary vegetation (“capoeiras”), so pollinators will also be protected and the maintenance of ecological service of pollination will ensure the supply of seeds for animals and humans. Pollinator friendly practices should be adopted by farmers, to support on-farm conservation, and increase Brazil nut production.

Grant: CNPq 556406/2009-05; Global Pollinator Project GEF/UNEP/FAO/MMA/FUNBIO

### PP-138

#### Honey Orchards with native forest species: an alternative to support Family Farming and the rehabilitation of degraded native forest in Chile

María Paz Molina<sup>1</sup>, Hernán Soto<sup>1</sup>, Jorge González<sup>1</sup>, Patricio Rojas<sup>2</sup>, Laura Koch<sup>1</sup>, Braulio Gutiérrez<sup>1</sup>, Roberto Ipinza<sup>3</sup>

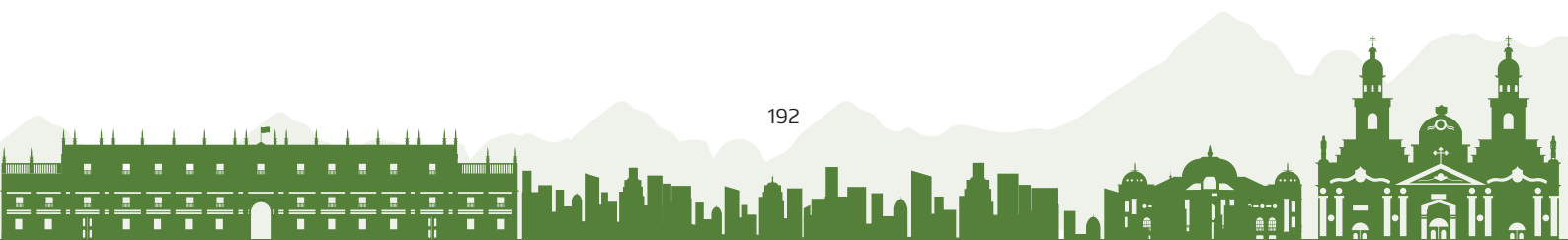
<sup>1</sup>Línea de Investigación Conservación y Mejoramiento Genético, Instituto Forestal, Sede Biobío, Chile

<sup>2</sup>Línea de Investigación Conservación y Mejoramiento Genético, Instituto Forestal, Sede Metropolitana, Chile

<sup>3</sup>Línea de Investigación Conservación y Mejoramiento Genético, Instituto Forestal, Sede Los Ríos, Chile

In Chile there are about 9 thousand beekeepers, who have between 6 and 277 hives each. The Biobío region is in fourth place in relation to the number of beekeepers (1,034), with an average of 85 hives per beekeeper. Of the total number of hives declared in Biobío, about 57% carry out transhumance, mainly due to the lack of flowers for bees and to provide pollination services for agriculture.

In relation to the above, the Conservation and Genetic Improvement team of the Forestry Institute of Chile (INFOR) is executing a program of the Regional Government of Biobío to increase the availability of honey flowers for beekeeping, through the establishment of honey orchards with an emphasis on species native forests. The program also provides technological tools to beekeepers to face climatic or other phenomena that affect the production of honey and its derivatives. The working model and the design of the honey orchards are applicable to other geographical regions of the country, involving various combinations of species to maintain the floral offer during the year, and also contributing to the restoration and biological connectivity of the native Chilean flora. The bases of the program and the results since its implementation are presented.



### PP-139

#### Native species from the Atlantic Forest that contribute to the production of *Apis mellifera* honey in the Quilombola Community of Pilões, Vale do Ribeira, São Paulo

Aline Lemos de Moraes<sup>1</sup>, Cristiane Bonaldi Cano<sup>2</sup>, Cynthia Fernandes Pinto da Luz<sup>1</sup>

<sup>1</sup>Laboratório de Palinologia PALINO-IPA, Instituto de Pesquisas Ambientais, São Paulo, Brazil

<sup>2</sup>Núcleo de Química, Física e Sensorial, Instituto Adolfo Lutz, São Paulo. Brazil

The Atlantic Forest has a vast richness of plant species and is considered a priority for conservation by the Brazilian government. About 21% of the remnants of this biome are in the Ribeira do Iguape Valley river, and due to it being one of the most biodiverse areas on the planet, in 1999 the United Nations Educational, Scientific and Cultural Organization (UNESCO) declared the region a Natural Heritage of Humanity. The region is home to traditional cultures that seek sustainable activities for their socioeconomic development, such as Quilombola Communities that have been practicing family beekeeping since 2003. With the aim of promoting honey production in the Quilombola Community of Pilões (24°29'08.5" S and 48°28'58.9" W), in Iporanga municipality, the certification of the botanical origin of six samples of centrifuged honey collected from *Apis mellifera* L. beehives located near native forest, during the main harvests from October 2019 to February 2023, was sought. The species occurring in the area that significantly contributed nectar to the honey were indirectly identified by melissopalynological analysis. The laboratory preparation of the samples followed the Classic European methodologies with and without acetolysis. The results showed that none of the samples were monofloral (with >45% of the counted pollen). In October 2019, the most frequent pollen types were *Allophylus*, *Protium* and *Tapirira*. In January 2021, there was a significant contribution from the nectar of native palms *Astrocaryum*, *Attalea/Butia*, *Euterpe/Syagrus* and the shrub *Heimia apetala*, and in January 2022, *Euterpe/Syagrus*, *Piptadenia* and *Heimia apetala* predominated. In February 2023, *Attalea/Butia* and *Euterpe/Syagrus* predominated. In a previous study conducted by the team in Pilões (2013 to 2015), *Astrocaryum*, *Euterpe/Syagrus* and *Protium* also made significant contributions, however, other pollen types presented higher frequencies, such as *Andira/Machaerium*, *Ilex*, *Matayba*, *Mikania cordifolia*, *Schinus* and *Serjania*. It is concluded that botanical origin certification is important to determine the relationship of the vegetation of the Atlantic Forest of the Ribeira do Iguape Valley river with the honey produced in Pilões, allowing for its greater appreciation in the market and better knowledge of Brazilian blooms.

### PP-140

#### How nectar resources affect pollinator assemblages

Maggie C Gill<sup>1</sup>, Keith F. A. Walters<sup>2</sup>

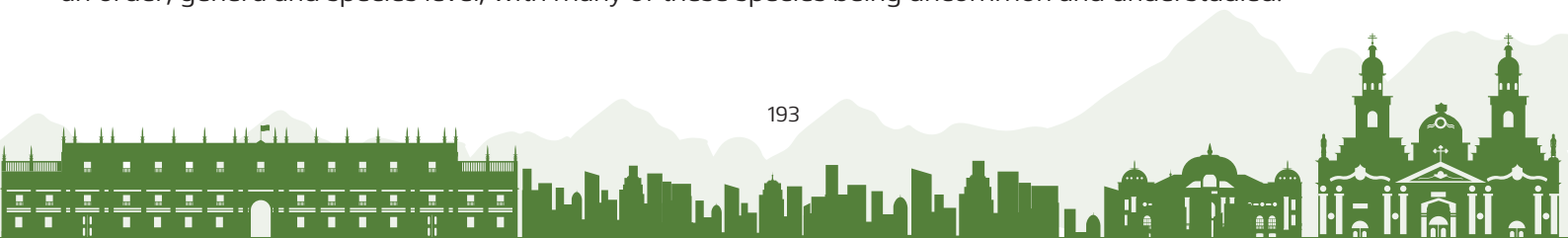
<sup>1</sup>Lennard-Jones School of Chemical and Physical Sciences Lennard-Jones Building Keele University Staffordshire ST5 5BG, UK

<sup>2</sup>Harper Adams University, Edgmond, Newport, TF10 8NB, UK

Pollinators provide a key ecosystem service which is ecologically and economically important to the United Kingdom. Widespread global declines have been reported in both pollinator species richness and abundance, which have been linked to similar declines in wild plant species richness and abundance. These declines have been attributed to factors such as landscape change, habitat loss, pests and diseases, pesticides and climate change. The UK government is addressing these declines through schemes such as the Nature Recovery Network and the National Pollinator Strategy.

This study investigated a broad spectrum of floral nectar resource available to UK pollinators, and identified nectar characteristics that affect pollinator foraging choices. The selection of flowers during foraging of pollinators from the orders Hymenoptera, Diptera and Lepidoptera was most significantly related to the percentage and volume of glucose present in the nectar offered by studied flowers, and to a lesser extent the percentage and volume of fructose and sucrose.

As nectar quality and quantity are both a plant species characteristic and primary drivers of pollinator forage choice the findings from this study will support policy makers in decision making on schemes to improve pollinator diversity and abundance. In particular it offers an insight into the nectar feeding preferences of a wide range of pollinators at an order, genera and species level, with many of these species being uncommon and understudied.



## Pollination and Bee Flora

### PP-141

#### Bee-lining in the bush: Locating wild honey bee nests in South Africa

Kaylin Kleckner, James D. Ellis

Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

The western honey bee, *Apis mellifera*, is intensely managed around the world as agricultural livestock. While modern beekeeping practices are essential for human food production, these practices do not reflect traditional beekeeping and honey-hunting techniques utilized with wild honey bees. *Apis mellifera* is native to parts of Europe, the Middle East, and Africa. In these regions, beekeepers commonly obtain their colonies by catching swarms or locating nest sites. Beekeepers looking for colonies and honey-hunters searching for honey to harvest use bee-lining techniques to locate wild honey bee nest sites. Bee-lining techniques include identifying foragers at a resource, such as water or blooming flowers, and following those satiated foragers back to their nest. Unmanaged colonies often dwell inside cavities in trees, the ground, rockfaces, or manmade structures. Locating nest sites is a historical beekeeping practice and the first step to answer a wide array of research questions regarding unmanaged honey bees. We utilized bee-lining techniques to locate wild and unmanaged colonies in the Eastern Cape, South Africa. We constructed and deployed stands that were stocked with dishes of honey near resources with foraging bees present. We followed foragers from natural or provided resources to their nest entrances. We recorded the location of nests and collected samples of workers to address future research questions regarding nesting behavior and ecology of wild honey bees in South Africa. Our work uses a traditional beekeeping practice to clarify how wild *A. mellifera* selects nest sites and interacts with other colonies in their environment.

### PP-142

#### Sustainable systemic indices in bee pollinating families

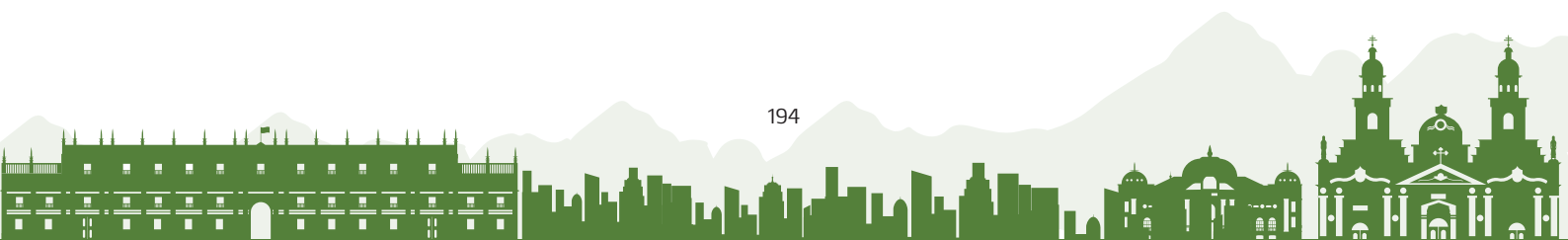
M<sup>a</sup> Loreto Fuertes Díaz<sup>1</sup>, Felisa Maria Hodgson Torres<sup>2</sup>, Carmen Celia López De Vergara Fuertes<sup>3</sup>

<sup>1</sup>Viceconsejería Sector Primario, Gobierno de Canarias, Santa Cruz de Tenerife, Spain

<sup>2</sup>Departamento de biodiversidad y conservación, Universidad de La Laguna, San Cristóbal de La Laguna, Spain

<sup>3</sup>Asociación Ecored, Santa Cruz de Tenerife, Spain

Agrarian systems are a complex reality. They constitute a mode of exploitation of the environment, with a durable trajectory based on systems of both natural and directed forces. The forms of agricultural production adapted to the bio-climatic conditions gives space respond to the natural conditions of the environment and the social needs of the place at a given time. The vision of productive activity as a systemic unit constitutes an instrument for developing a field of knowledge integrated into the different elements, including the territorial environment where the activities developed by farmers and stockbreeders are generated. The research studies based on the General Systems Theory (GST), try to give a scientific form of approximation and representation of a reality, it is applicable to the activity of the agro-livestock companies. It is a technique that is applied to agro-rural systems with a multifunctional vision, a useful tool for the design, establishment and management of sustainable agriculture. It is a scientific paradigm characterized by a holistic vision of value, integrated into a whole. Both the environmental movement and the scientific-academic movement have managed to reach a general consensus on the importance of addressing the problem of sustainable development from a global and multidisciplinary viewpoint. With this, it is possible to approach it as systems of measurement and to identify the problems surrounding the sustainability of the rural world and to seek buffer systems for them. We study the potential of bees in the productive phase as a means of increasing the results of the exploitation activity, guaranteeing the conservation of the environment of the farms. For this purpose, it is necessary to provide indicators to monitor the system as a management tool for the agro-apiculture families and their caretakers. This study proposes to incorporate in the field notebooks, the necessary elements to conserve, in good health, the pollinator families and thus favor their conservation and sustainability using a systematic methodology.



### PP-143

#### Development of honey bee colonies kept in two-entrance hives for Italian zucchini pollination in greenhouses

Mateus Augusto Marques Batista<sup>1</sup>, Gustavo Alan Siniscalchi<sup>2</sup>, Cássia Regina de Avelar Gomes<sup>2</sup>, Tainá Angelica De Lima Freitas<sup>2</sup>, Aline Yukari Kato<sup>2</sup>, Yara Martins Molina Ferraz<sup>2</sup>, Thaís Regina Ramos Alves<sup>2</sup>, Daniel Nicodemo<sup>1</sup>

<sup>1</sup>Department of Animal Science, São Paulo State University (Unesp), College of Agricultural and Technological Sciences, Dracena, Brazil.

<sup>2</sup>Post Graduate Program in Animal Science, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil.

Fully closed greenhouses prevent the access of pollinators to this growing area, a fact that can limit fruit production of crops dependent on biotic pollinators. The aim of the study was to assess whether the use of hives with two entrances mitigate the harmful effects on the development of honey bees colonies submitted to confinement without decreasing the production of Italian zucchini (*Cucurbita pepo*) cultivated in fully closed greenhouses. Italian zucchini plants were cultivated in two fully closed greenhouses (15x7 m), in which Langstroth five frame nuclei with honey bee colonies were introduced. In one greenhouse, the hives were completely confined while in the other the bees could visit zucchini flowers from 5 to 9 am and during the rest of the day they were directed to the external environment to forage on other plants, as these colonies were installed on two-entrance hives. Each hive remained in one of the greenhouses for 15 days, with successive replacements until the end of zucchini flowering period. We evaluated the visitation behavior and the effect of bee visits to flowers on fruit production. Bee population development was studied by mapping the comb areas with brood and food before and after each pollination period. The bee visitation frequency did not change over the 14 days with bees managed in hives with two entrances. With total confinement, there was a reduction in the visitation of pistillate and staminate flowers from the eighth and fourteenth days, respectively. Fruit set and fruit weight were not influenced by the type of confinement. Total confinement of bees resulted in reductions 0.6 and 14.2 times greater than partial confinement, respectively for food and brood areas. There was no significant difference in the brood area observed between the beginning and the end of each cycle for colonies installed in hives with two entrances. Partial confinement met the pollinator demand of the zucchini crop and was less harmful to the honey bee colonies compared to the fully confined colony management.

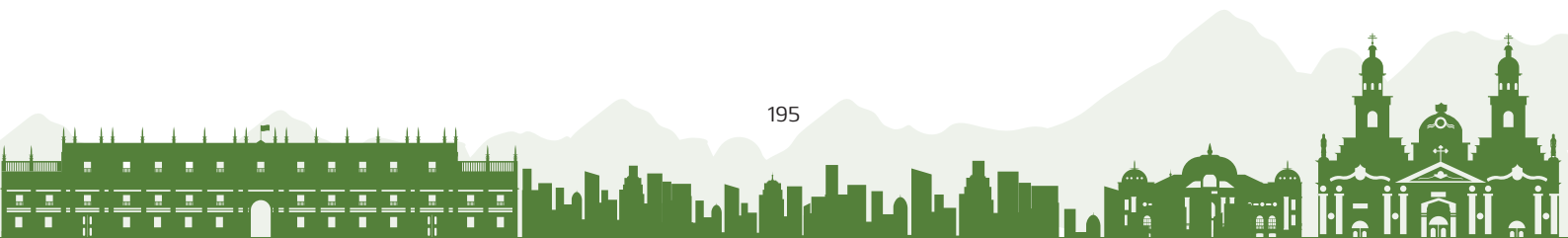
### PP-144

#### Floral resource used by Africanized bees *Apis mellifera* sp. Linnaeus, 1758 and stingless bees, *Melipona fasciculata* Smith, 1854 and *Scaptotrigona postica*, Latreille, 1807, at São Luís School Farm (FESL) - UEMA, Brazil

Eleuza Gomes Tenório, Luciana Dos Santos Aires, Monique Hellen Martins Ribeiro

Zootecnie Department, State University Of Maranhão, Brazil

The pollen spectrum present in honey is very diversified, due to the potential of the Brazilian flora. Understanding the plant resources used by bees is important both for honey production and for preserving the flowering and management of bees in natural, agricultural and urban environments. The objective was to determine the floral origin of honey stored in colonies of *Apis mellifera* sp. (Africanized), *Melipona fasciculata* (tiúba) and *Scaptotrigona postica* (tubi), present at São Luís School Farm (FESL - UEMA), based on the morphology of the pollen grains found in the honey samples. Data were collected monthly between February 2017 and January 2018 and submitted to the acetolysis method. 102 pollen types were found in the honey samples. The most representative species observed in the samples of *A. mellifera* sp. were: *Alternanthera brasiliana* (F. *Amaranthaceae*) (April - 56%) and *Eucalyptus* sp. (F. *Myrtaceae*) (June - 45.71%); in the samples of *M. fasciculata* were: *Turnera ulmifolia* (F. *Turneraceae*) (January - 73.77%), *Protium* sp. (F. *Burseraceae*) (March - 62.27%), *Mimosa caesalpinifolia* (F. *Fabaceae*) (April - 64.58%) and *Alternanthera brasiliana* (F. *Amaranthaceae*) (August - 91.47%); in the samples of *S. postica* were: *Mimosa caesalpinifolia* (F. *Fabaceae*) (December - 47.81% and November - 36.44%), *Neptunia plena* (L.) Benth. (F. *Fabaceae*) (January - 41.56%) and *Alternanthera brasiliana* (F. *Amaranthaceae*) (September - 36.07%). *A. mellifera* sp. and *M. fasciculata*, shared 24 pollen types; *A. mellifera* sp. and *S. postica*, shared 13 pollen types and *M. fasciculata* and *S. postica*, shared 18 pollen types. Only in the month of August, *A. mellifera* sp., *M. fasciculata* and *S. postica* shared three plant species concomitantly: *Protium* sp. (F. *Burseraceae*), *Eucalyptus* (F. *Myrtaceae*) and *Borreria verticillata* (F. *Rubiaceae*). 50 exclusive pollen types were found for bee species, being for *A. mellifera* (37 species), *M. fasciculata* (8 species) and *S. postica* (5 species). Despite the study area being in an urban area, 102 pollen types were identified, among native, introduced and cultivated plants, as well as different sizes. This demonstrates that FESL - UEMA has a preserved reserve area, making it possible to raise different bee species.





### PP-145

#### Lacunas Bees: a tool to facilitate the identification of data gaps of bees in Brazil

Kátia Paula Aleixo<sup>1</sup>, Ana Lúcia Assad<sup>1</sup>, Sidnei De Souza<sup>2</sup>, Dora Canhos<sup>2</sup>

<sup>1</sup>Associação Brasileira de Estudos das Abelhas (A.B.E.L.H.A.), São Paulo, Brazil

<sup>2</sup>Centro de Referência em Informação Ambiental (CRIA), Campinas, Brazil

The expansion of knowledge about the richness and distribution of bees is essential for their conservation. Since 2016, A.B.E.L.H.A. and CRIA have been working on the development and digital dissemination of information about bees, using speciesLink network (<https://specieslink.net/>) and Moure's Bee Catalogue (<http://moure.cria.org.br/>) as reference systems. The system "Knowledge gaps of bees in Brazil" (<http://moure.cria.org.br/lacunas/>) was developed by comparing Moure's Bee Catalogue data on species that occur in Brazil with data available through the speciesLink network. The results include the number of records for all species cited in Moure's Bee Catalogue separated in groups: (1) without records, (2) with 1-5 records, (3) with 6-20 records and (4) with more than 20 records. The system also highlights species data gaps per state. Users may search for subfamilies, scientific names or endangered species or may follow a taxonomic path until genus and select a species. Reports are available for subfamilies, tribes, genus, and species. For example, selecting bee subfamilies that occur in Brazil and using the most inclusive search option, the Lacunas' report of January 2023 indicates that, for the 1,965 species listed in Moure's Bee Catalogue, 703 species (36%) do not have any data record in speciesLink. Considering the absence of records in relation to the total number of species in each subfamily, almost half of the known species of Colletinae still does not have any data record in speciesLink (47% of 126 cataloged species). In the other subfamilies, the absence of records in the network was distributed as follows: Halictinae (45% of the 337 cataloged species), Andreninae (43% of 121 species), Megachilinae (38% of 350 species) and Apinae (30% of 1031 species). At the species level, the report also presents the conservation status, besides the distribution according to Moure's Bee Catalog and speciesLink. Associated with each map, there is a link to the original information. This tool helps define strategies to reduce or eliminate gaps, such as new collecting and digitization efforts, inviting new collections to the network, and promoting specialist training in the identification of understudied bee groups.

### PP-146

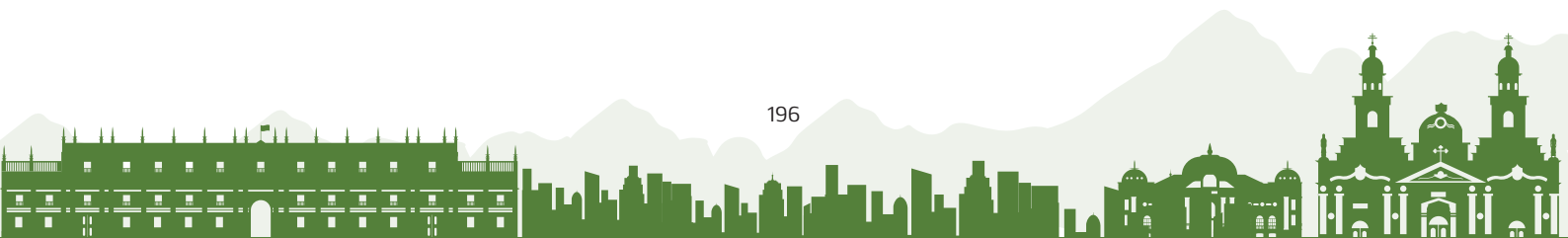
#### Applied pollination in Brazilian coffee crops reduce the peaberry formation and increase the cup quality

Diego Moure-Oliveira<sup>1</sup>, Joyce Mayra Volpini Almeida Dias<sup>1</sup>, Ensei Uejo Neto<sup>2</sup>, Carlos Pamplona Rehder<sup>1</sup>, Guilherme Jorge Gomes Sousa<sup>1</sup>, Andresa Berretta<sup>1</sup>, Andresa Berretta<sup>1</sup>

<sup>1</sup>AgroBee, Ribeirão Preto, SP, Brazil

<sup>2</sup>Ensei Neto Educação, São Paulo, SP, Brazil

The food production industry and market are undergoing a transformation. Agricultural activities have been operating differently from the past, notably due to the technological, scientific and social progress currently achieved. All these advances will gradually make this sector more profitable, safe, efficient and sustainable. Coffee beans are one of the most important commodities on the world and Brazil is the main world producer, with more than 2 million hectares planted; and several Brazilian coffee farms are searching for innovative solutions to improve their production, allying environmental, social and economic sustainability. One of those solutions is the use of managed honeybees to supplement the contributions of wild pollinators. During the 2021/22 harvest, 17 coffee farms were studied in Brazilian southeast region. Coffee crops that receive managed honeybees significantly reduce the peaberry formation in 17.3% ( $t = 3.9$ ,  $df = 15$ ,  $p < 0.01$ ). High temperatures during the blooming period can denature pollen grains and compromise the fertilization of the ovules, so despite being autogamous, the Arabica coffee can benefit from cross-pollination performed by bees, because these pollinators can take external pollen to those flowers that suffered from these high temperatures, reducing the peaberry formation and increasing the productivity. Other point, the reducing of the fecundation period due to the presence of honeybees visiting flowers during the blooming period can promote a more homogeneous ripening fruits during the harvest period, improving and cup quality. The Binomial test showed that coffee drinks that receive the applied pollination treatment significantly reduced the astringency flavor in the cup (number of successes = 1, number of trials = 17,  $p < 0.01$ ). Almost 60% of the control coffee samples – without applied pollination – presented astringency in the sensory analysis of the drink. The presence of astringency in coffee cup is due to the presence of unrip fruits and it is a characteristic that reduces the cup quality. These results showed that the applied pollination can contribute to a sustainable coffee production, increasing the coffee yield and quality.



### PP-147

#### Africanized honey bees as biological inputs for orange production

Matheus Portela Pinho<sup>1</sup>, Joyce Mayra Volpini Almeida Dias<sup>1</sup>, Diego Moure Oliveira<sup>1</sup>, Richard Lopes Raposo<sup>1</sup>, Carlos Pamplona Rehder<sup>2</sup>, Guilherme Jorge Gomes Sousa<sup>1</sup>, David De Jong<sup>3</sup>, Andresa Berretta<sup>4</sup>

<sup>1</sup>AgroBee Solutions in Pollination and Sustainability, Ribeirão Preto, Brazil

<sup>2</sup>AgroBee Solutions in Pollination and Sustainability, Ribeirão Preto, Brazil; Novo Mel Industry and Foreign Trade Ltd., São Paulo, Brazil

<sup>3</sup>Department Genetics, Ribeirão Preto Medical School, University of São Paulo. Ribeirão Preto, Brazil

<sup>4</sup>AgroBee Solutions in Pollination and Sustainability, Ribeirão Preto, Brazil; Apis Flora Industrial and Commercial Ltd., Ribeirão Preto, Brazil

Plants and bees have co-evolved for mutual benefit. When pollinators visit flowers, they transfer pollen from one flower to the female part of another, ensuring fertilization and fruit formation. Cross-pollination is an important evolutionary adaptation of plants, allowing for new combinations of hereditary factors and increasing the production of fruits, seeds, and others. During the flowering period, orange blossoms are highly attractive to bees, which collect the abundant and rich nectar, and consequently carry out pollination. The flowering period of Southeast Brazilian orange orchards occurs between July - November, with harvesting between February - June, depending on the variety and region. Cross-pollination is beneficial for orange production, and its absence can have a significant impact on fruit production; however growers and agronomists are often not aware of the importance of pollination. When the orange blossoms were in the pre-anthesis phase, five Africanized honey bee colonies were placed in the orchard per hectare, each with 5 - 7 frames of brood, respecting all safety protocols. There was a pollinated area and a control area within the same farm; both had the same orange variety and the same soil conditions. A control area was marked 500m away from where the beehives were installed for comparison. The hives remained in the orchard for about 20 days, and after 80% of the flowers were pollinated, the beekeepers removed the beehives in order to allow pest control measures. During harvest, an estimate of the crop was made, with 27,000 trees being harvested in the pollinated area and 34,262 in the control area, showing a mean of 568 fruits per tree in the bee treatment area and 442 fruits per tree in the control area. There was a significant increase of 22.18% in fruit per plant (students t value = 3.72, df = 5, p < 0.05). Based on these results, the managers requested a larger number of bee colonies for the next season. These results demonstrate the importance of maintaining bees in the orchard during flowering and implementing agricultural practices that promote cross-pollination to help ensure maximum fruit production, including temporary suspension of pesticide applications.

### PP-148

#### Framework for identifying priority zones for monitoring and assessment of wild populations under climate change

Andre Luis Acosta<sup>1</sup>, Charles Fernando Santos<sup>2</sup>, Vera Lucia Imperatriz Fonseca<sup>3</sup>, Ricardo Caliarí Oliveira<sup>4</sup>, Tereza Cristina Giannini<sup>5</sup>

<sup>1</sup>Instituto Tecnológico Vale. Rua Boaventura da Silva, 955, 66055-090, Belém, Pará, Brazil

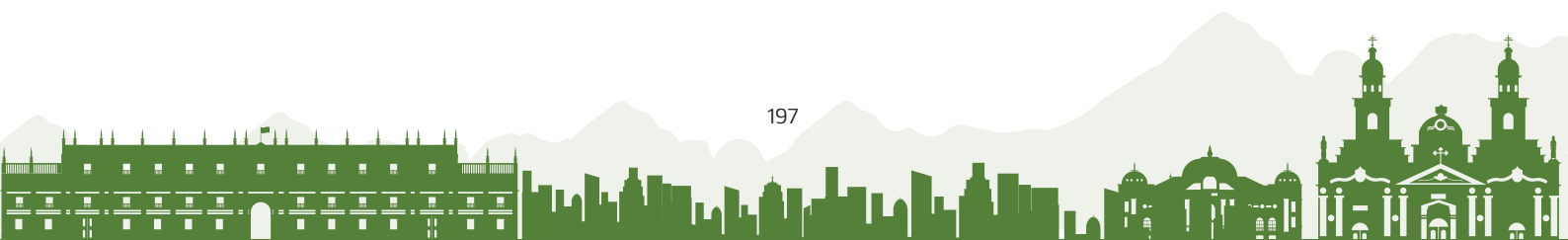
<sup>2</sup>Escola de Ciências da Saúde e da Vida, Programa de Pós-Graduação em Ecologia e Evolução da Biodiversidade, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90610-000, Porto Alegre, Rio Grande do Sul, Brazil.

<sup>3</sup>Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14, n.321, 05508-090, São Paulo, Brazil.

<sup>4</sup>Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia - Universitat Autònoma de Barcelona.

<sup>5</sup>Universidade Federal do Pará. R. Augusto Correa, 1, 66075-110, Belém, Pará, Brazil.

Climate change is affecting wild populations worldwide, and assessing the impacts on these populations is essential for effective conservation planning. The integration of advanced analytical techniques holds promise in providing detailed spatially explicit information on climate change impacts on wild populations, providing fine-grained metrics on current environmental quality levels and trends of changes induced by estimated climate change scenarios. Here, we propose a framework that integrates three modern approaches aiming to designate the most representative zones for long-term monitoring, considering different levels of climate pressures from estimated climate change scenarios for two coming periods. We assume that greater the variation of future climatic conditions from their original state, greater the impacts on populations. Similarly, we also assume the opposite: lower level of combined variation, lower effects. Therefore, from our perspective, it becomes a priority to monitor species in zones presenting these extremes to evaluate population at major and minor impacts from climate change. To determine these zones, we developed a framework by integrating three analytical approaches: Species Distribution Modeling, Principal Component Analysis and Generalized Procrustes Analysis. We used the SDM to determine the climatically persistent suitable extent for species, the geographic extent where the climate will remain suitable for the survival of species populations today and in all future scenarios considered. By using a PCA, we sliced the persistent suitable extent into inner belts based on the variability of extremes and averages of meaningful climate variables. Subsequently, we measured, analyzed, and described the climatic variability and future trends in each inner belt by applying GPA approach. We tested our framework with a climatically sensible Neotropical stingless bee species as study case, *Melipona fasciculata*. Our results showed that the framework adds significant analytical advantages for priority area selection for population monitoring. Most importantly, it allows a robust discrimination of areas where climate change will exert greater-to-lower impacts on the species. We show that the results provide superior geospatial design, qualification, and quantification of climate change effects than frequent SDM approaches usually provide. These improvements increase assertiveness and precision in determining priority areas, reflecting in better decision-making for conservation and restoration.



### PP-149

#### Effect of temperature and relative humidity on the visitation of bees (*Apis mellifera* L.) in *Prunus salicina* Lindl. plum flowers. cv Leticia in orchards with and without the use of anti-hail screens

Tânia Patrícia Schafaschek<sup>1</sup>, Simone Silmara Werner<sup>2</sup>, André Amarildo Sezerino<sup>3</sup>

<sup>1</sup>Agricultural Research and Rural Extension Company of Santa Catarina, Videira Experimental Station, Videira-SC, Brazil

<sup>2</sup>Departament of Informatics and Statistics (INE), Federal University of Santa Catarina (UFSC), Florianópolis-SC, Brazil

<sup>3</sup>Agricultural Research and Rural Extension Company of Santa Catarina, Caçador Experimental Station, Caçador-SC, Brazil

The anti-hail screen offers protection to orchards. However, little is known about their effect on the foraging activity of bees, and consequently on pollination. In this work, the flight activity at the beehive entrance and visitation behavior of *Apis mellifera* bees on plum blossoms cv Leticia and their correlation with climate variables were evaluated. The experiment was carried out in a completely randomized design, in the 2018 to 2020 harvests, in the municipality of Videira, SC. The treatments consisted of the use of black anti-hail screen with 18% shading, plus the control, without screen. The number of bees going in and out of the beehive per minute and the number of flowers visited by bee, observing 30 bees for 1 minute each and the number of bees per leg (main branch), counting the number of bees that arrived at the leg during the timed period of one minute, was evaluated. Temperature and humidity data were obtained using data loggers and also provided by Epagri/CIRAM. Analysis of variance (F test) was carried out considering the effects of crop and coverage presence. The assumptions of the parametric model were verified using the Bartlett and Shapiro-Wilk tests and the Box-Cox transformation was used when necessary. Spearman's correlation coefficient was used to verify the existence of correlation between variables. The analyzes were carried out with the aid of the R environment considering the 5% significance level. Air temperature and humidity did not differ between treatments. The departure of bees from the hives showed a positive correlation with temperature (p-value 0.0254) and the number of flowers visited (p-value 0.0047), the number of bees per leg (p-value 0.0116), the entrance (p-value 0.0039) and departure of bees from the hives (p-value 0.0029) showed a negative correlation with relative humidity. The departure of bees from the hives showed a harvest effect (p-value < 0.0001) (27.65±3.97 and 49.05±2.4 for the 2019 and 2020 harvests) and treatment (p-value 0.0349) (55±7.05 and 35.79±3.42 for treatments without and with screen, respectively). The anti-hail screen did not interfere the visitation of flowers by the bees. It had an influence on their flight activity.

### PP-150

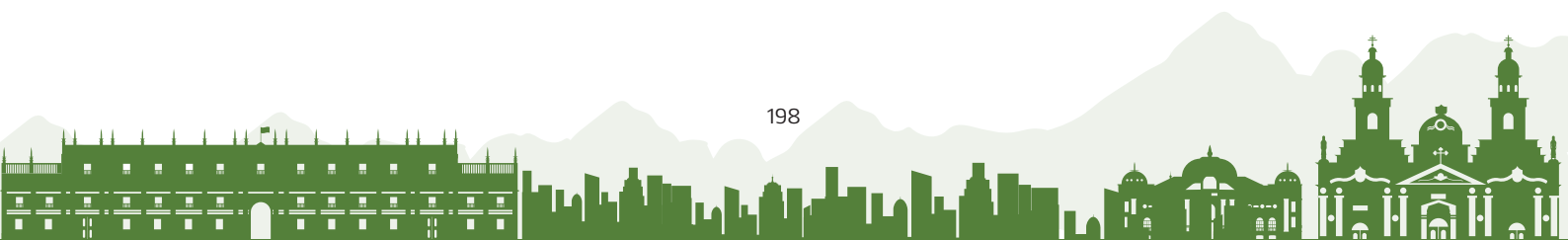
#### Bee diversity increases fruit set and yield quality of strawberry (*Fragaria x ananassa*) cultivated in the neotropics

Breno M. Freitas<sup>1</sup>, Pedro De Assis De Oliveira<sup>1</sup>, Larysson Feitosa Dos Santos<sup>1</sup>, Marcelo Casimiro Cavalcante<sup>2</sup>

<sup>1</sup>Setor de Abelhas, Departamento de Zootecnia, Universidade Federal do Ceará, Fortaleza - CE, Brasil

<sup>2</sup>Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Redenção - CE, Brasil.

The expansion of temperate crops to tropical environments where their natural pollinators are not found but there is a great diversity of floral visitors demands investigations on how this greater biodiversity of potential pollinators is related to yield in these crops. Thus, we investigated the diversity of bee visitors to strawberry (*Fragaria x ananassa*), cultivar San Andreas, cultivated in Tianguá, CE, in NE Brazil, where we evaluated their foraging behavior, fruit setting rate, fruit weight, fruit quality and size in diameter, according to the Common Market of the South (Mercosul), resulting from a single visit of five bee species in comparison to open pollination (OP), spontaneous autopollination (SA) and manual cross-pollination (MCP). The results showed 19 species of bees visiting the strawberry flowers, the vast majority belonging to the Apidae family (69%), followed by Halictidae (21%), Andrenidae (5%) and Colletidae (5%). The most abundant were *Scaptotrigona* aff. *depilis* (Sad) which visited flower between 6am-5pm, *Apis mellifera* (Am) from 7am-3pm, *Trigona recursa* (Tr) between 6am-4pm, *Plebeia flavocincta* (Pf) between 8:00h -15:00h and *Nannotrigona* aff. *testaceicornis* (Nat) between 9:00h-15:00h. In general, all the bees touched the reproductive structures of the flowers and pollen was the most collected resource. There were differences  $H=101.6832$  ( $p < 0.01$ ) in the setting rate, with SA setting significantly less fruits than the other treatments, and in the weight of strawberries ( $p < 0.01$ ) which ranged from 6.36g (SA) to 11.68g (MCP) and 13.58g (OP). All bee species produced strawberries as heavier as MCP, while Tr, Pf and SA differed from OP. The treatments Tr and SA did not produce fruits in size 3 (diameter >30mm), while size 2 (between 20 and 30 mm) was the most frequent in all treatments. The treatments also varied in relation to the percentage of marketable fruits: Nat = 92%, OP = 92%, Am = 61%, MCP = 61%, Tr = 36%, Sad = 33% and Pf = 25%, SA = 13%. It is concluded that except for Nat, the studied bees cannot individually maximize the production or quality of the fruits, requiring their complementary actions to produce the best results, as observed in OP.



### PP-149

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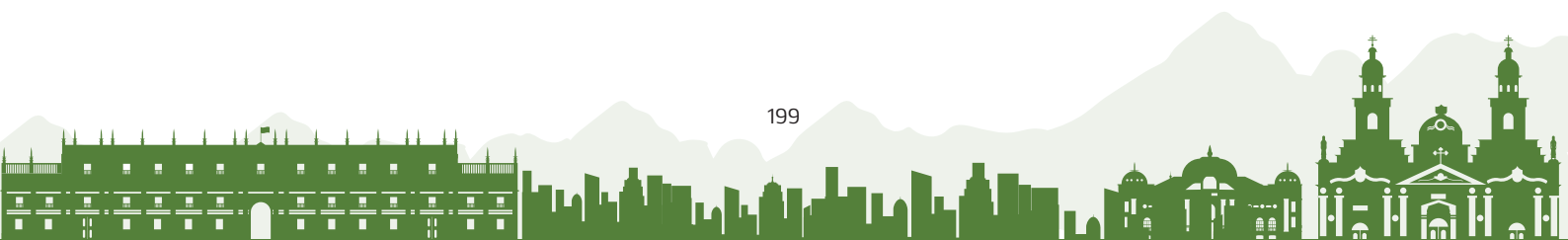
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## Pollination and Bee Flora

### PP-151

#### Applied pollination service in soybean crops on Brazilian farms

Matheus Portela Pinho, Diego Moure Oliveira, Joyce Almeida, Andresa Aparecida Berretta, Andresa

Aparecida, Andresa Aparecida Berretta

AgroBee – Berretta Eleve Pesquisa & Desenvolvimento, Ribeirão Preto/SP;

Brazil is one of the world's top producers of soybeans, with more than 135 million tons produced during the 2020/21 harvest. Soybean is a self-pollinating plant but cross-pollination by pollinators such as honeybees (*Apis mellifera*) can increase its yield. A study was conducted during the 2021/22 harvest, in which managed beehives were installed in soybean crops between the stages R2 and R3 at a rate of 2 beehives per hectare. The study was performed on four farms, with three different soybean cultivars, BRS 284, BRS 511, and Brasmax Zeus. The yield observed in each treated area was compared to their respective control areas, which were in the same plot but 250 meters away from the beehives. During the harvest period, five to six sampling points were randomly plotted in both pollinated and control areas for each farm. The results showed that all farms observed an increase in yield in the pollinated area, with a difference of 20.5% for BRS 284, 18.2% for BRS 511, and 5.1% and 17.2% for the two farms of Brasmax Zeus cultivar. The increase in yield was likely due to the applied pollination helping soybean plants avoid the abortion of flowers and pods, resulting in a greater number of beans per plant. This study's results demonstrate that applied pollination services in soybean crops can be an important and environmentally sustainable activity in the agricultural system, helping to meet the growing demand for food production worldwide.

### PP-152

#### From species to landscape: how to estimate food resources for pollinators?

Jacek Jachuła<sup>1</sup>, Aleksandra Splitt<sup>2</sup>, Mikolaj Boranski<sup>2</sup>

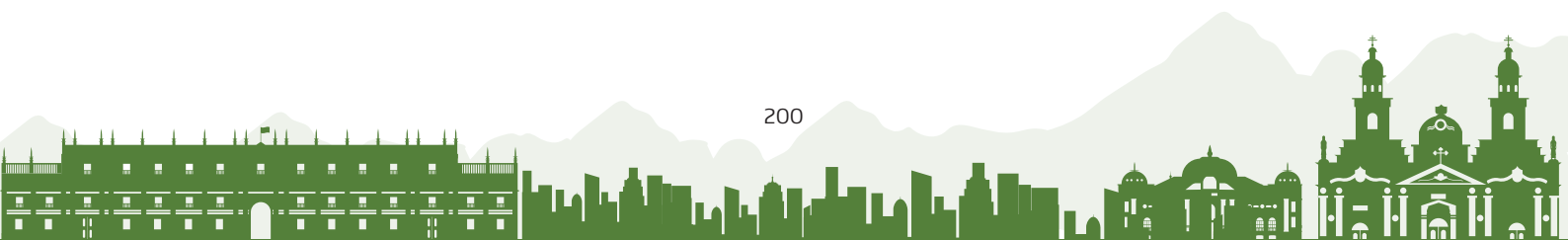
<sup>1</sup>Department of Botany, Mycology and Ecology, Institute of Biological Sciences, Maria Curie-Skłodowska University in Lublin, Poland

<sup>2</sup>Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

One of the strategies to support pollinators in agricultural landscapes is to protect/enhance habitats that are rich in nectar- and pollen-producing flora. However, literature data on the quantity of food resources in different types of habitats is scarce, not to mention that it is not recognized at landscape level. The reason for that could be time- and labour-consuming analyses and lack of a well-established method. Here we present a step by step method for estimation of food resources for pollinators. It can be summarized as follows:

1. recognition of pollinator-friendly habitats (selection of habitat types and/or sub-types for detailed studies)
2. assessment of flora composition in each type of habitat (list of species with their cover)
3. selection of plant species for study on sugar and/or pollen production
4. recording species flowering phenology and abundance
5. quantification of sugar and/or pollen production at species level
6. determination of sugar and/or pollen resources at habitat level
7. recognition of habitat coverage within landscape
8. quantification of sugar and/or pollen resources at landscape level

A case study from south-eastern Poland was used to illustrate the method. General food resources were calculated. However, our approach can be also used when calculating food available for specific insect species provided that their dietary requirements and population densities are known.





## Pollination and Bee Flora

### PP-153

#### Red mason bee (*Osmia bicornis*) avoid creating single-component pollen provisions from mass flowering crops in favor of trees' pollen

Mikolaj Boranski<sup>1</sup>, Aleksandra Splitt<sup>1</sup>, Jacek Jachula<sup>2</sup>

<sup>1</sup>Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

<sup>2</sup>Department of Botany, Mycology and Ecology, Institute of Biological Sciences, Maria Curie-Skłodowska University in Lublin, Poland

In an agricultural landscape, bees are exposed to a number of stress factors. One of them is the nutritional stress associated with the reduces the diversity of available pollen sources and increases the likeliness of pollen diet homogeneity. This situation can occur in large-scale monocultures like rapeseed. Nutritional stress can disturb bees' development and presumably impact on their phenotype. One of the most commonly use measures of development instability is fluctuating asymmetry. We hypothesized that the low pollen diversity in larval provision of polylectic bee *Osmia bicornis* affects its development. To verify the content of larval provision we performed a palynological analysis (pollen composition, diversity, percentage of dominant pollen type, content of *Brassica napus* and *Quercus* pollen in the provisions). Afterwards we modelled how these pollen traits impact wing size and asymmetry. Results of palynological analysis showed that *O. bicornis* do not form single-component pollen provisions from rapeseed pollen. Quite the contrary the content of anemophilous trees pollen, especially *Quercus* was relatively high. The pollen quality of larval provision has an influence on the size of wings, but not on the fluctuation asymmetry in *O. bicornis*. Our results support earlier reports that wing size reflects response to nutritional stress better than wing asymmetry. This research was funded by the Ministry Of Science And Higher Education as a part of the statutory activities of the National Institute of Horticultural Research in Skierniewice (task number ZP/4/2020 - 8.4.20).

### PP-154

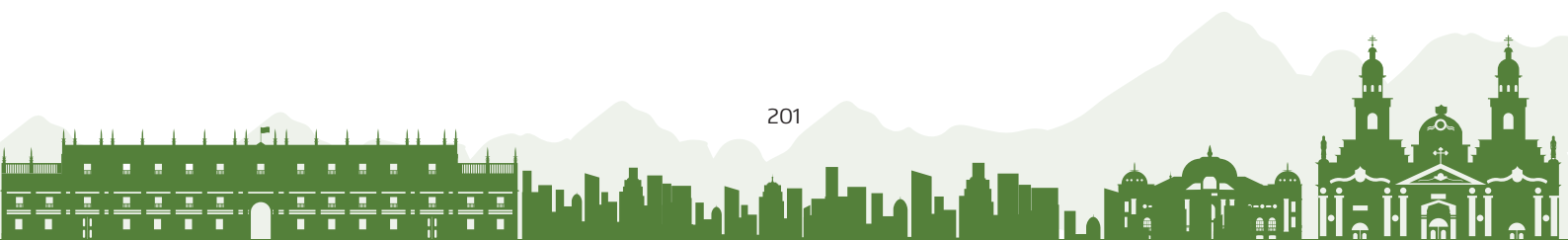
#### Creating habitats for bees in an intensive agricultural area

Asger Søgaard Jørgensen, Lise Hansted

The Danish Beekeepers Association

Denmark is a small country of only 44.000 km<sup>2</sup>. Occupying 62 % of the area, agriculture is dominating the Danish landscape. 92% of this area is plowed each year and fields sizes are still increasing leaving very few habitats for pollinating insects. For many years our focus has been on gardens, parks, hedgerows, field corners and roadsides as habitats for pollinators in such a landscape. This is still the case.

Spread over the Danish landscape we also have more than 2000 cemeteries and 186 golf courses with great potential to become valuable habitats for insects and with a huge potential for establishing showcases to the public as many people visit these places. Many of the cemeteries and some of the golf courses have abandoned the use of pesticides as well as fertilizers in order to create more natural vegetation. They have actively been looking for ways to increase the biodiversity with focus on use of indigenous plants and establishment of sanctuaries for insects. We will present our project "Be Bee kind" with examples of activities on cemeteries and golf courses, as well as the results of an inventory from our local golf course where we found a total of 46 species of bees and 102 species of plants valuable for bees. It means more than 15 % of the bee species ever registered in Denmark including some rare species where registered from the golf course.



### PP-155

## Non-compliance with the World Trade Organization Agreements by exporters of the European bumblebee, *Bombus terrestris*

Cecilia Smith Ramirez<sup>1</sup>, Adriana Estefanía Rendón Funes<sup>2</sup>, Mario Leiva<sup>3</sup>, Marina Arbetman<sup>4</sup>, Marcelo Aizen<sup>5</sup>, Luis Agüero<sup>6</sup>

<sup>1</sup>Universidad de Los Lagos, Institute of Ecology and Biodiversity (IEB), Universidad Austral de Chile

<sup>2</sup>Institute of Ecology and Biodiversity (IEB), Museo de Historia Natural Alcide d'Orbigny

<sup>3</sup>Universidad de Los Lagos

<sup>4</sup>Universidad Nacional del Comahue

<sup>5</sup>Universidad Nacional del Comahue, Wissenschaftskolleg zu Berlin

<sup>6</sup>Isla Butachauques, Sector Nayahue Alto, s/n, comuna de Quemchi

International companies commercially rear bumblebees worldwide, the trade of which is regulated through agreements established by the World Trade Organization (WTO). Scientific studies have shown multiple negative effects of introduced commercial bumblebees on native bees in Japan, Australia, Sweden, Israel, Chile and Argentina, calling into question exporting countries' compliance with some of the established WTO international sanitary regulations. We analyzed international WTO sanitary regulations focusing on the international trade of bumblebees from the European Union and Israel, especially regarding bumblebee exports to Chile and their side effects in neighboring Argentina. We have gathered evidence showing that exporters of bumblebees do not comply with WTO international trade agreements in at least two ways: (1) the quality of commercialized bumblebees differs from the quality declared in their certifications, and (2) the countries that sell the bumblebees violate sanitary agreements, producing negative effects on other native pollinating insects and causing a cascade of negative effects affecting both the environment and agriculture. This suggests that companies that raise bumblebees are currently in breach of WTO regulations and continue to contribute to major environmental damage in southern South America and elsewhere.

### PP-156

## Trees friendly to bees

Decio Luiz Gazzoni

Researcher, Embrapa Soybean, Brazil

Our book "Plants that pollinators like", launched in 2023, is part of the larger framework of integration of agricultural activities aiming at sustainable agriculture. The book has more than one thousand pages and eight hundred illustrations, containing texts on pollination carried out by bees; indications of actions for the conservation of bees; basic notions of Apiculture and Meliponiculture; a list of more than 2,000 species of bees that exist in Brazil; a list with a detailed description of more than 200 plants that pollinators like; and an array of about 2,500 plants and the species of bees that visit them. The information and recommendations of the book will allow for improving the floristic recomposition of native vegetation in rural properties; the occupation of margins roads and highways areas with species favorable to pollinators; the urbanization of urban parks, gardens, and the planting of essences in streets or gardens, backyards, aiming to pollinator protection. It is in line with the recognition of the paramount importance of the ecosystem service of pollination in the global effort for the production of food and other agricultural products. Farmers can use the information to provide friendly places for bees to nest, shelter, and collect resources, such as pollen, nectar and others. The book will also be useful for urban planners and support to policymakers at city halls, state, and the federal government, to provide favorable environments for pollinators. Citizens can also use the information to plant trees in their gardens, backyards, and leisure areas. Presently, Brazilian growers have annual incomes estimated at around US\$80 billion attributed to natural pollination. With additional initiatives favoring pollinators with plants that offer shelter and resources for their food and reproduction, the benefit can be even greater, representing larger margins for farmers and lower prices for consumers. And society as a whole will benefit from rural and urban landscapes that favor biodiversity.



### PP-157

### Characterization of the Flora Surrounding Apiaries Based on the Palynological Analysis of Honeys for Commercial Purposes from the Municipality of Casablanca, Región de Valparaíso, Chile

Patricio Vásquez Quitral<sup>1</sup>, Denisse Carvajal<sup>2</sup>, Oscar Fuentes Molina<sup>3</sup>, Andrés Vargas Fernández<sup>4</sup>, Eduardo Castañeda Zúñiga<sup>5</sup>

<sup>1</sup>Escuela de Medicina Veterinaria y Agronomía, Universidad de Las Américas, sede Providencia, Santiago, Chile.

<sup>2</sup>Laboratorio de Innovación, Empresa Biopacific, Santiago, Chile.

<sup>3</sup>Departamento de Política Sectorial, Oficina de Estudios y Políticas Agrarias (ODEPA), Santiago, Chile.

<sup>4</sup>Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile, Avda. Santa Rosa 11315, La Pintana, 8820808 Santiago, Chile.

<sup>5</sup>Fundación Instituto Profesional Duoc UC, Santiago, Chile.

In recent years there has been a considerable increase in the consumption of natural and less processed foods by people, which has encouraged the development of techniques to assess the quality of these type of products. In the case of the nectar honey, it is formed from the foraging carried out by the honey bees (*Apis mellifera*) to obtain nectar and pollen from the flora surrounding the hives. However, honey bees are able to sense the quantity and quality of forage resources available in the sector, to achieve greater efficiency in the food gathering process.

For its part, the palynological analysis is the study of the composition of pollens found in honeys and that allows the identification of the botanical and geographical origin of this type of product. Being able to recognize the botanical composition of honey, makes it possible to reveal the identity of what the consumer is really acquiring when buying honey. In addition, the realization of palynological analysis by specialized laboratories allow to give added value to the final product, particularly to those honeys that may be considered monofloral or bifloral. In this context, we performed a study of characterization of nectar honeys, harvested in the Casablanca Valley - Región de Valparaíso (Chile), carried out through traditional palynological analysis and diversity indexes (Simpson y Shanon-Weaver). The results showed the presence of 14 native species (7 of them endemic to Chile) and 16 of introduced species, in 7 of honey samples. There was a 64% coincidence in the botanical origin, previously declared by the beekeeper, compared to the result of the palynological analysis of these samples. The result of the total pollen grains in honeys evidenced a Simpson diversity index of 0.87, while the Shanon-Weaver index was 2,557. Despite the fact that one or two type of pollen (vegetal specie) was mostly found in some honey samples (nectar honeys, monofloral), the results of the palynological analysis suggests that honey bees were capable of foraging numerous species (among 13-18 species).

### PP-158

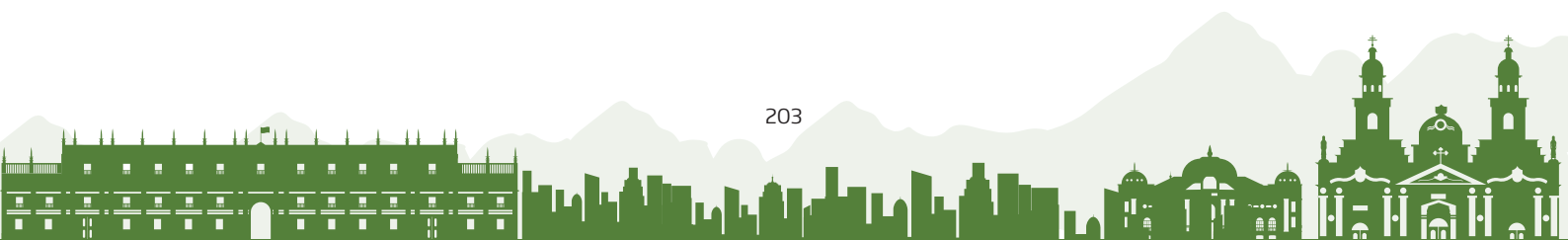
### Do floral traits of buzz-pollinated plants and the characteristics of the visiting bee species explain the pattern of compartmented interactions?

José Mesquita-Neto<sup>1</sup>, Clemens Schindwein<sup>2</sup>

<sup>1</sup>Departamento de Biología y Química, Facultad de Ciencias Básicas, Universidad Católica del Maule, Talca, Chile

<sup>2</sup>Departamento de Botânica, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Pollen from poricidal anthers is predominantly extracted by bees. The relationships of plant species with poricidal anthers and bees, however, are rather heterogeneous and include different subgroups of bee species that remove pollen legitimately through floral sonication as well as species that collect pollen illegitimately. Although plants with poricidal anthers share a specialized mechanism of pollen release, their pollinators tend to be dissimilar. Benefiting from the great functional and taxonomic diversity of plant species pollinated by vibration in the tropics, we aimed to understand which floral traits of plants with poricidal anthers are associated with the physical and behavioral characteristics of respective flower-visiting bee species. We measured the rates of stigma-contacts, conspecific plant visits per time, flower handling-time and intertegular span and noted the pollen extraction mode of the bees visiting the flowers of eight species with poricidal anthers in co-occurrence and co-flowering. On the plants' side, we estimated the pollen amount per flower and plant, the floral display area, and the number of flowers per plant. We found no strong correlations between floral display size and pollen amount among plant species, indicating independent forces that influence pollinator preferences. The higher quantity of pollen per flower and per plant was correlated with the longer handling times of the bees per flower and plant individual, respectively. Sonicating bees visited more flowers and plants per time and contacted stigmas fivefold than other bees. The species with the highest number of pollen grains per flower, was also the species with the most flower visits of vibrating and non-vibrating bees, being, therefore, preferred by potential pollinators but also by pollen thieves. Despite plant species with poricidal anthers sharing the same pollen release mechanism, variations in floral morphology and the physical and behavioral characteristics of the visitors contribute to explaining the compartmentation of their interactions.



### PP-159

### Plants, people, and pollinators: insights from this biotic interaction in latin america

Rubem Avila Jr<sup>1</sup>, Marina Basualdo<sup>2</sup>, Natalia Arbulo<sup>3</sup>, León Felipe Cano-Medina<sup>4</sup>, Ariel Angel Cespedes Llave<sup>5</sup>, **Adriana Correa-benitez**<sup>4</sup>, Leonardo Galetto<sup>6</sup>, Ariel Muñoz Navarro<sup>7</sup>, Guiomar Nates Parra<sup>8</sup>, Agustín Saez<sup>9</sup>, Sheena Marie Salvarrey<sup>10</sup>, Carlos Silva Neto<sup>11</sup>, Yannet Quispe Delgado<sup>12</sup>

<sup>1</sup>Laboratório de Pesquisas em Interações Ecológicas, Universidade Federal do Pampa, campus São Gabriel, RS, Brazil

<sup>2</sup>PROANVET- Facultad de Ciencias Veterinarias. Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Argentina

<sup>3</sup>Centro Universitario Regional del Este- CURE, Universidad de la República de Uruguay, UdelaR

<sup>4</sup>Departamento de Medicina y Zootecnia de Abejas, Conejos y Organismos Acuáticos (DMZ: ACyOA), Facultad de Medicina Veterinaria y Zootecnia (FMVZ), Universidad Nacional Autónoma de México (UNAM)

<sup>5</sup>Instituto Experimental de Biología "Luis Adam Briangon"- Facultad de Ciencias Químico Farmacéuticas y Bioquímicas. Universidad de San Francisco Xavier de Chuquisaca, Bolivia

<sup>6</sup>Facultad de Ciencias E., F. y Naturales, Universidad Nacional de Córdoba (IMBIV) y CONICET, Argentina

<sup>7</sup>Instituto de Geografía, Pontificia Universidad Católica de Valparaíso PUCV, Chile

<sup>8</sup>Pesquisadora colaboradora de Solatina, Colombia

<sup>9</sup>Universidad Nacional del Comahue y CONICET, Argentina

<sup>10</sup>Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

<sup>11</sup>Instituto Federal de Educação, Ciencia e Tecnologia de Goiás (IFG), GO, Brazil

<sup>12</sup>Huarango Nature, Lima, Peru

Different aspects of human culture are associated with plants, which have been used as food, medicine, or making different tools since the beginning of humanity. In this way, maintaining the diversity of plant species is essential for the subsistence of aboriginal and farmer communities in Latin America. Pollination service becomes relevant because the pollinators ensure the seed and fruit sets to both maintain plant populations and nourish people. We evaluated the pollinators, pollination, and reproduction for plant species used by Latin-American people through a systematic review of the literature. Reproductive system, floral biology, and pollinators data were gathered. Pollinators were taxonomically grouped and people were categorized as native, traditional communities and family farmers. We found 87 plant species belonging to 30 families. Myrtaceae (10 spp), Fabaceae (06 spp.), Arecaceae (05 spp.), and Passifloraceae (05 spp.) were the most representative families. A narrow pollination niche performed by a single pollinator functional group was prevalent in the plant register (almost 50 % of species) (Chi-square = 42.011, df. - 3, p < 0.001), and in this specialized group, 90 % of plant species were pollinated by bees. Our findings highlight that although 50 % of species within this pollinator functional group were associated with native and exotic bee species. Native bees were associated exclusively with 40 % of plants, while the exotic "Apis mellifera" was the exclusive pollinator of 10% of the plants recorded. Native people use a higher number of plants (44 species) than the other two groups. About 40 % of the species are used in common by the native and traditional communities, and only 11% of plant species were shared among the three human groups. Plants exclusively utilized by native people and traditional communities presented a high proportion of specialized pollination systems (55 % and 80 % of species respectively). Our results indicated that although there are diversified pollination systems in the plant species most used by Latin-American people, the prevalence of bees as pollinators must be highlighted. Active policies should be applied to protect pollinators, particularly for bees, and improve food sovereignty and biocultural conservation in Latin America.

### PP-160

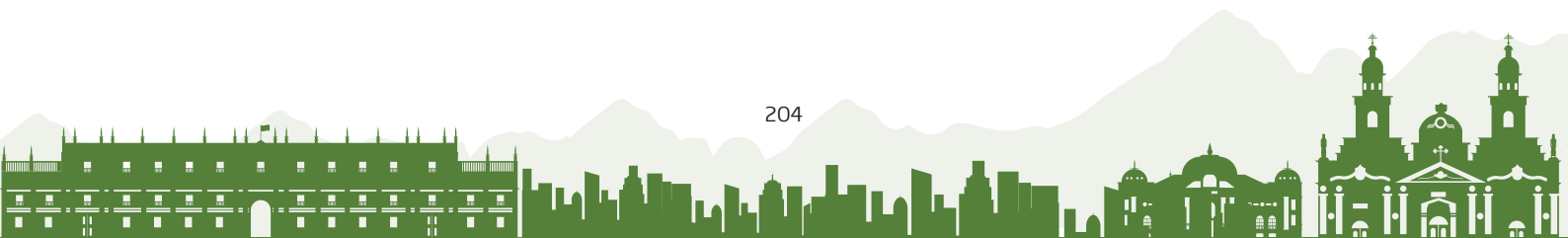
### Automatic acoustic recognition of bee species using Deep-Learning models

**José Neiva Mesquita-Neto**<sup>1</sup>, Alef Iury Siqueira Ferreira<sup>2</sup>, Nádia Felix Felipe Da Silva<sup>2</sup>, Fernanda Neiva Mesquita<sup>2</sup>, Thierson Couto-Rosa<sup>2</sup>, Víctor Hugo Monzon<sup>1</sup>

<sup>1</sup>Instituto de Informática, Universidade Federal de Goiás, Goiânia, Goiás, Brazil

<sup>2</sup>Laboratorio Ecología de Abejas, Departamento de Biología y Química, Facultad de Ciencias Básicas, Universidad Católica del Maule, Talca, Chile.

Bees capable of performing floral sonication (or buzz-pollination) are among the most effective pollinators of blueberries. However, the quality of pollination provided varies greatly among species visiting the flowers. Consequently, the correct identification of flower visitors becomes indispensable to distinguishing the most efficient pollinators of blueberry. However, taxonomic identification normally depends on microscopic characteristics and the active participation of experts in the decision-making process. Moreover, the many species of bees (20,507 worldwide) and other insects are a challenge for a decreasing number of insect taxonomists. To overcome the limitations of traditional taxonomy, automatic classification systems of insects based on Machine-Learning (ML) have been raised for detecting and distinguishing a wide variety of bioacoustic signals, including bee buzzing sounds. Despite that, classical ML algorithms fed by spectrogram-type data only reached marginal performance for bee ID recognition. On the other hand, emerging systems from Deep Learning (DL), especially Convolutional Neural Networks (CNNs), have provided a substantial boost to classification performance in other audio domains, but have yet to be tested for acoustic bee species recognition tasks. Therefore, we aimed to automatically identify blueberry pollinating bee species based on characteristics of their buzzing sounds using DL algorithms. We designed CNN models combined with Log Mel-Spectrogram representations and strong data augmentation and compared their performance at recognizing blueberry pollinating bee species with the current state-of-the-art models for automatic recognition of bee species. We found that CNN models performed better at assigning bee buzzing sounds to their respective taxa than expected by chance. However, CNN models were highly dependent on acoustic data pre-training and data augmentation to outperform classical ML classifiers in recognizing bee buzzing sounds. Under these conditions, the CNN models could lead to automating the taxonomic recognition of flower-visiting bees of blueberry crops. However, there is still room to improve the performance of CNN models by focusing on recording samples for poorly represented bee species. Automatic acoustic recognition associated with the degree of efficiency of a bee species to pollinate a particular crop would result in a comprehensive and powerful tool for recognizing those that best pollinate and increase fruit yields.





### PP-159

### Plants, people, and pollinators: insights from this biotic interaction in latin america

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### PP-160

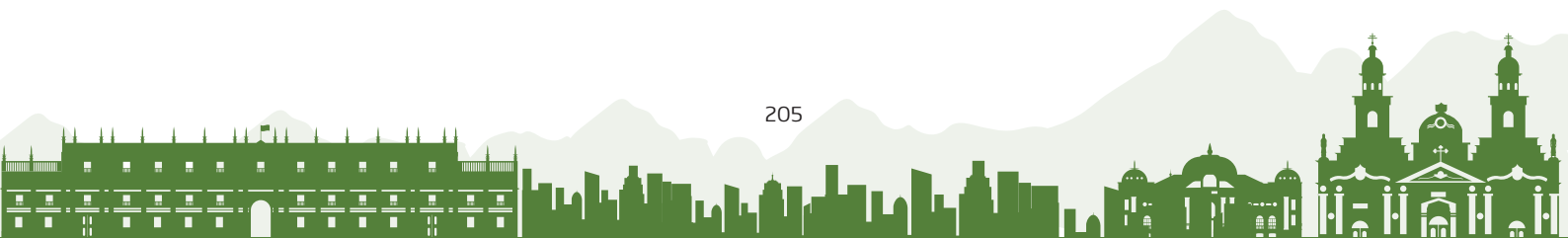
### Automatic acoustic recognition of bee species using Deep-Learning models

**José Neiva Mesquita-Neto**<sup>1</sup>, Alef Iury Siqueira Ferreira<sup>2</sup>, Nádia Felix Felipe Da Silva<sup>2</sup>, Fernanda Neiva Mesquita<sup>2</sup>, Thierson Couto-Rosa<sup>2</sup>, Víctor Hugo Monzon<sup>1</sup>

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### PP-161

#### Quantification of pollen grains on the body of floral visitors in canola crop

Luciano Marinozzi, Soledad Camila Villamil, Ana Andrada

Laboratorio de Estudios Apícolas (LabEA-CIC), Departamento de Agronomía, Universidad Nacional del Sur (UNS). Bahía Blanca, Prov. Buenos Aires, Argentina

The number of pollen grains that pollinators deposit on flowers during each visit depends on the morphological characteristics of the flower and the insect, as well as their behavior; furthermore, it can vary widely among taxa. The amount of pollen on the bodies of the main floral visitors of canola, *Brassica napus* L., in the central-southern region of Buenos Aires was evaluated during 2019 in experimental plots at INTA-Barrow (-38.319305, -60.239380). Three groups were analyzed: honey bees (*Apis mellifera* L.), *Campsomeris bistrimaculata*, and halictids, identified in previous studies as the main floral visitors. To determine the amount of pollen grains on the insects, specimens were individually washed in a solution composed of 40 ml of distilled water and 0.2 g of Tween 20. Before washing the honey bees, the nectar foragers and pollen foragers were differentiated, and the third pair of legs was removed since corbicular pollen is not available for pollination. Three milliliters of the washing solution were placed in vials containing the individuals and vortexed for 10 minutes. The insect body was removed and the remaining solution was centrifuged for 10 minutes at 2500 rpm. The supernatant was discarded, and distilled water was added to the precipitate to complete a volume of 1 ml. Four aliquots were taken to count the pollen grains in a Neubauer chamber of 0.9 mm<sup>3</sup>. Male *C. bistrimaculata*, with an average of 10 439 ( $\pm 7 292$ ) pollen grains, carried the least amount of pollen ( $p < 0.05$ ). There were no statistical differences between pollen-foraging honey bees (26 497  $\pm$  13 324 grains/bee), nectar-foraging honey bees (21 514  $\pm$  13 947 grains/bee), halictids (24 139  $\pm$  15 136 grains/bee), and female *C. bistrimaculata* (19 078  $\pm$  12 028 grains/wasp). Female *C. bistrimaculata*, *A. mellifera*, and halictids carry more canola pollen on their bodies than male *C. bistrimaculata*; however, all of them could fulfill the role of canola pollinators.

### PP-162

#### Attractiveness of indigenous extracts on abundance and diversity of pollinators and honey bee visitation in sunflower

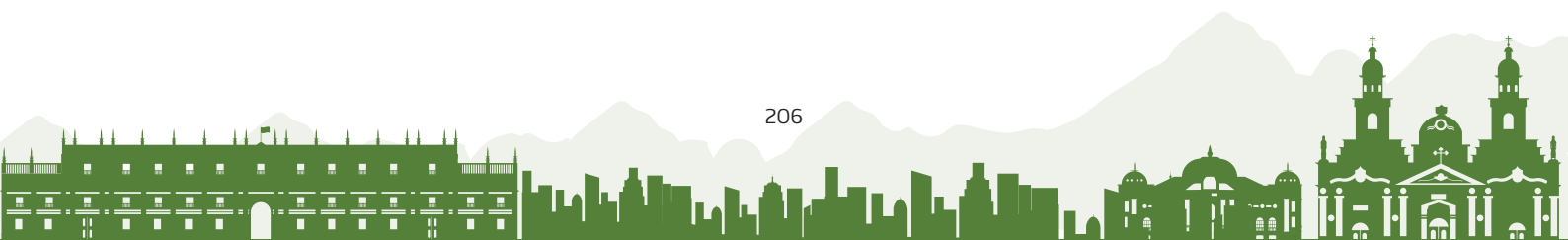
Badariprasad P. R.<sup>1</sup>, Sanganna Sajjan<sup>2</sup>, Sreenivas A. G.<sup>3</sup>

<sup>1</sup>Badariprasad P. R. Assistant professor, Department of Agricultural Entomology, College of Agriculture, Gangavathi, UAS, Raichur. Karnataka INDIA

<sup>2</sup>Sanganna Sajjan Scientist, Department of Agricultural Entomology, MARS, Raichur

<sup>3</sup>Sreenivas A. G Professor and Head, Department of Entomology, College of Agriculture, Raichur

The field experiments were carried out during monsoon and autumn 2020-21 to identify the influence of native attractants on the diversity of sunflower pollinators, bee visitation, and yields of sunflowers at ARS, Gangavathi, and MARS, Raichur. The results revealed that the number of species recorded was higher in Gangavathi, with 16, compared to Raichur (14 species) during both seasons, implying the higher richness in Gangavathi. The evenness index is higher in both sites implying less evenly distributed pollinators. Shannon-Wiener Index values of 2.23 and 2.21 were recorded in Gangavathi plots during the monsoon and autumn seasons, respectively, and 2.11 for Raichur plots during both seasons, implying that thus sunflower field of Gangavathi is more diverse compared to Raichur. A higher number of visitors were recorded in plots sprayed with basil oil which is on par with a commercial attractant, i.e., profuze sprayed plots, followed by citronella oil, mint oil, lemon grass oil, sugar solution in both ARS, Gangavathi, and MARS, Raichur sunflower plots at first and second spray during monsoon and autumn seasons. Yields of sunflowers followed a similar trend as that of bee visitation.



### PP-163

#### Effect of honey bees *Apis mellifera* and other insect pollinators on the quantity and quality of sunflower *Helianthus annuus* seeds in a subtropical environment

Francisca Contreras Escareño<sup>1</sup>, Raúl Ávalos Silva<sup>2</sup>, Jose Octavio Macias-Macias<sup>2</sup>, José María Tapia González<sup>2</sup>, Alvaro De la Mora<sup>3</sup>, Ernesto Guzman-Novoa<sup>3</sup>

<sup>1</sup>Departamento de Producción agrícola, Centro Universitario de la Costa Sur, Universidad de Guadalajara, Jalisco, México

<sup>2</sup>Departamento de Ciencias de la Naturaleza, Centro Universitario del Sur, Universidad de Guadalajara, Jalisco, México

<sup>3</sup>School of Environmental Sciences, University of Guelph, Guelph, Ontario Canada

Sunflower *Helianthus annuus* is a crop of global importance but most of the studies on the effect of pollinators on this crop have been conducted in countries of temperate climates. The objective of this study was to evaluate the impact of honey bees *Apis mellifera* and other insect pollinators on sunflower yield in a subtropical environment in Southern Jalisco, Mexico. Nine 2 x 2 x 2 m cages made with thrip mesh that prevented the entrance of insects, were evenly distributed over sunflower plants in a 2-ha field planted with the crop. The plants in those cages served as the non-insect pollinated treatment, whereas a similar area of plants (not covered with mesh) was marked to serve as the insect pollinated treatment. To provide sufficient pollination, six hives containing honey bee colonies were evenly distributed in the field. During the blossom time, visiting insects were collected with a net from the flowers once a week in eight different occasions, and later identified using entomological keys. Besides honey bees, 13 species of native wild bees were identified, including *Centris varia*, *Centris nítida*, *Ceratina* spp., *Lasioglossum* spp.1, *Lasioglossum* spp.2, *Evylaeus* spp., *Augochlora nigrocyanea*, *Augochora* spp., *Peponapis utahensis*, *Exomalopsis* spp., *Diadasia rincois*, *Protandrena* spp., and *Dialictus* spp. Additionally, three species of wasps, *Polybia occidentalis*, *Campsomeris ephippium*, and *Thiphia* spp., as well as seven species of hemipterans, *Leptoglossus clypealis*, *Nezara viridula*, *Acanthocephala femorata*, *Largus* spp., *Dysdercus mimulus*, *Rhyparochromidae*, and *Apiomerus* spp. were identified. Moreover nine species of beetles were also identified, including *Cerambycidae*, *Cotinis mutabilis*, *Paranomala* spp., *Diabrotica* spp.1, *Diabrotica* p.2, *Diabrotica* spp.3, *Caligrapha*, *Diptotaxis* spp., and *Coccinelidae*. There were significant differences between treatments ( $p < 0.05$ ). Higher values for variables such as weight of sunflower heads, number of seeds per flower, seed weight, and number of fertilized seeds were observed in the plants with open access to pollinators. It is concluded that pollinators like honey bees, wild bees, and numerous other insects contributed to increase the quality and quantity of sunflower seeds. It is important to preserve the existence and abundance of local pollinators of sunflowers in subtropical environments.

### PP-164

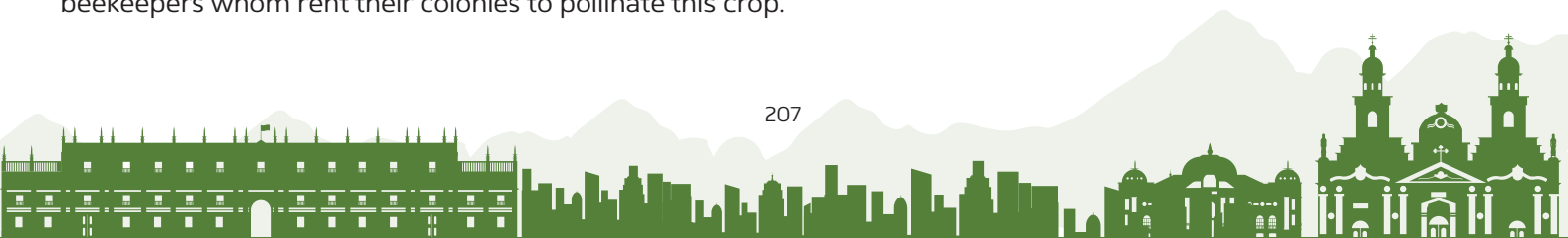
#### Beneficial impact of pollination services by honey bees *Apis mellifera* in raspberry *Rubus idaeus* fields in Southern Jalisco, Mexico

Jose Octavio Macias-Macias<sup>1</sup>, José María Tapia González<sup>1</sup>, Francisca Contreras Escareño<sup>1</sup>, Ernesto Guzman-Novoa<sup>2</sup>, Alvaro De la Mora<sup>2</sup>

<sup>1</sup>Universidad de Guadalajara. Centro de Investigaciones en Abejas CIABE. Departamento de Ciencias de la Naturaleza. CUSUR. México.

<sup>2</sup>University of GUELPH. Honey Bee Research Center. School of Environmental Sciences. Canada.

The use of bees for pollination services increases crop production as well as fruit size, quality, and yield. In Southern Jalisco, Mexico, the cultivation of berries (raspberries, blueberries, blackberries, and strawberries) is increasing exponentially, but growers know little about the benefits of using bees to pollinate their crops. Therefore, the objective of this study was to demonstrate the beneficial effects of honey bee pollination in raspberry *Rubus idaeus* fields. Four hives with honey bee colonies were evenly distributed in a 1 ha raspberry field just before plants bloomed. Two treatments were tested: 1) a treatment without pollinators, which consisted of 10 groups of 40 plants each, that were caged with Agribon® fabric to prevent bees from pollinating the flowers, and 2) a treatment with pollinators, which consisted of similar 40-plant groups that were labeled, but not caged. The fruits were harvested when they were ripe ( $n=950$ ), and the following variables were measured: weight (g), polar diameter (mm), equatorial diameter (mm), number of drupelets and sweetness (brix degrees). Significant differences were found between the two treatments for all the variables compared ( $p < 0.05$ ). In all cases, the treatment with open pollination resulted in higher values for all the variables measured, with differences ranging from 127% to 372%. These results demonstrate that the use of honey bees to pollinate raspberry plants positively impacts fruit quality and the economy of growers, as well as that of beekeepers whom rent their colonies to pollinate this crop.



## Pollination and Bee Flora

### PP-165

#### Honey bee pollination service improves sunflower yield and production quality

Bojan Stipešević<sup>1</sup>, Zlatko Puškadija<sup>1</sup>, Marin Kovačić<sup>1</sup>, Bojana Brozović<sup>1</sup>, Karolina Tucak<sup>1</sup>, Clémence Riva<sup>2</sup>, Fabrice Requier<sup>2</sup>, Anto Mijić<sup>3</sup>, Branimir Šimić<sup>3</sup>, Luka Andrić<sup>3</sup>

<sup>1</sup>Faculty of Agrobiotechnical Sciences Osijek, University of Osijek, Osijek, Croatia

<sup>2</sup>Université Paris-Saclay, CNRS, IRD, UMR Évolution, Génomes, Comportement et Écologie, France

<sup>3</sup>Agricultural Institute Osijek, Osijek, Croatia

The importance of pollination service of insects has been important topic over the last decades, due to the pollinator declines driven by biotic pressures and environmental changes such as global warming and pesticides exposure. In order to investigate importance of honey bee (*Apis mellifera*) to sunflower pollination, we conducted an experiment in five sites in Northeastern Croatia during year 2022. We investigated whether honey bees visiting sunflowers affect yield and oil content as production quality indicator. For that, we designed an experiment with following treatments: CT) open pollination with free access of pollinators to flowers; TI) insect pollination has been prevented by net caps during whole flowering period; HP) only hand pollination has been conducted, whereas net caps disabled insects from pollination, HO) hand pollination and open pollination were conducted, and SE) net caps were removed from sunflowers during 10 minutes period twice during flowering stage, and pollinators were recorded during visits. On each site, five groups of five sunflowers, set up 10 m apart among themselves, were marked and subjected to described treatments at the beginning of flower opening stage, one treatment per one marked sunflower's head. Net caps were removed after flowering stage, and marked sunflowers were picked up at full maturity. Sunflower head diameter, number of fertile and sterile grains, mass of fertile grains, grains moisture and oil content were determined for each head, and grain yield has been recalculated from plant's density and each head grain's mass. Results showed that the most observed flower visitors were honey bee (more than 97%). Open pollination treatment (CT) yielded the highest head diameter, total number of fertile grains, mass of grains and oil content, followed by treatments HO and SE, whereas treatments without pollinators (HP and TI) recorded up to 18% smaller head diameter, 32% less fertilized grains, 35-39% smaller grain mass and 3-7% less oil content. Grain yield achieved by open pollination treatment (CT) was 5,7 t/ha, whereas denying pollinators visit to sunflower caused grain yield reduction of 35-39%. This study, conducted as the part of the SafeAgroBee project showed the importance of honey bee as crop pollinators.

### PP-166

#### A contribution to the botanical study of honeys produced in the Jerte Valley (MW Iberian Peninsula)

Jesús Baena Sánchez, Estefanía Sánchez Reyes, David Rodríguez de la Cruz

Department of Botany and Plant Physiology, University of Salamanca, Spain

The pollen profile of 15 honeys from the natural region of Valle del Jerte (Extremadura, MW Iberian Peninsula) and 2 honeys from nearby areas were studied. Four of these honeys were extracted in spring and the rest in late summer of years 2019 and 2020.

The aim was to determine the botanical and geographical characteristics of these honeys and, on the basis of the data obtained, to evaluate the capacity and quality of bee production in the area. These studies were carried out using the relevant quantitative and qualitative palynological methods (González-Porto et al., 2018).

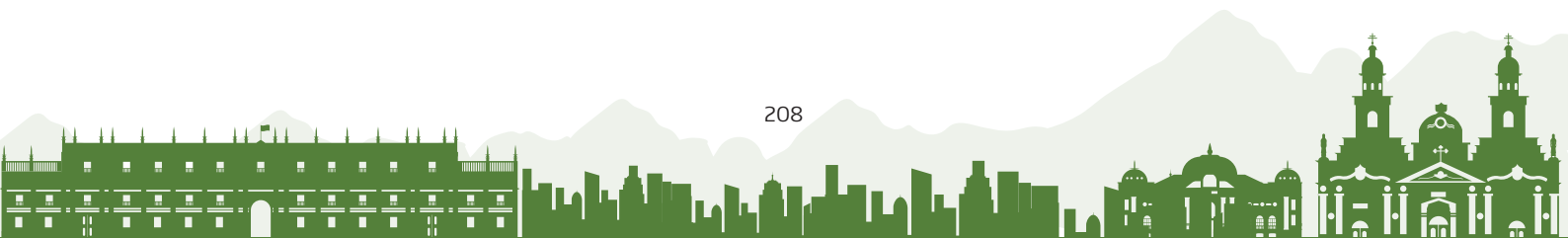
The quantitative analysis showed a medium and medium-high content of botanical elements, with an average number of 453,903 botanical elements in 10 g of honey. 12 of these samples fall into Maurizio's Class III while the remaining 5 are in Class IV. The qualitative analysis showed similar pollen richness values, typical of the flora of the Jerte Valley, with a high percentage of chestnut (*Castanea sativa* Mill) pollen grains. An electrical conductivity of less than 0.8 mS/cm was detected in 4 samples, while the rest exceeded this figure, suggesting a production from honeydew elements, according to European regulations (Council Directive 2001/110/EC).

Taking into account this physico-chemical parameter and the palynological evaluation, 4 samples were considered as "polyfloral", 2 as "chestnut honey" and 11 as "honeydew honey" or "forest honey". Finally, certain geographical markers that characterise the region and honey production have been defined based on the flora and climatic conditions of the environment.

#### References:

Council Directive 2001/110/EC of 20 December 2001 relating to honey.

González – Porto, A. V., Sánchez Reyes, E., De Linares Fernández, C., Rodríguez de la Cruz, D., Sánchez Sánchez, J., Belmonte Soler, J., Seijo Coello, M. C., Valencia Barrera, R. M., García Rogado, M. R., y Matías Martínez, Y. (2018, octubre 25 – 27). "El Grupo de Trabajo de la Miel y la normalización de protocolos de análisis melisopalínológicos". IX Congreso Nacional de Apicultura, Tenerife, España.



### PP-167

#### Resilience and adaptation to climate change: a qualitative analysis of beekeeping in Chile

Héctor Arrieta<sup>1</sup>, Ariel Muñoz<sup>1</sup>, Karen Yáñez<sup>2</sup>, Germán Zapata<sup>1</sup>, Martina Gajardo<sup>1</sup>, Enrique Rojas<sup>1</sup>, Ana López<sup>1</sup>

<sup>1</sup>Centro de Acción Climática, Universidad Católica de Valparaíso, Valparaíso, Chile.

<sup>2</sup>Centro de Biotecnología Dr. Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Valparaíso, Chile.

Climate change is one of the most pressing challenges facing society today, because it is a phenomenon that pushes the development of adaptation strategies to better face the impacts associated with changes in climate. Studies have shown that there is a close relationship between climate change and its effects on beekeeping in Chile, a sector that plays a very important role for food security and the economy in rural areas. In this context, the objective of this study was to evaluate which factors or characteristics could be determinants for the resilience of beekeepers in the face of Climate Change and, how these factors are reflected in the adaptation measures implemented to face the challenges related to climate change and reduce the impacts on beekeeping. For this, semi-structured interviews and surveys were conducted with around 1,000 beekeepers throughout Chile. The information collected from surveys was divided into two dimensions: the social dimension, in which aspects of the beekeeper's experience, training and other details were addressed; and the climatic dimension, which included the perception of climate change and its impacts on beekeeping. Additionally, the results were complemented with a bibliographic review of other studies carried out in different parts of the world.

### PP-168

#### Friend or foe? Some flowers may offer large amounts of pollen but at the same time pose a heavy metal poisoning threat to pollinators – preliminary study

Jacek Jachula<sup>1</sup>, Aleksandra Splitt<sup>2</sup>, Mikołaj Borański<sup>2</sup>, Karolina Tymoszuk<sup>1</sup>, Agnieszka Nawrocka<sup>3</sup>

<sup>1</sup>Department of Botany, Mycology and Ecology, Institute of Biological Sciences, Maria Curie-Skłodowska University in Lublin, Poland

<sup>2</sup>Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

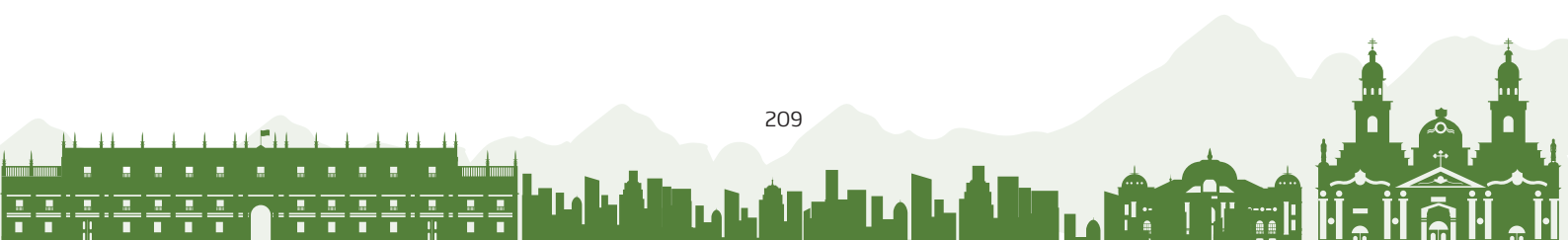
<sup>3</sup>Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland

Non-cropped plant species can provide substantial amounts of pollen to pollinating insects before and after mass-flowering crops (e.g. winter rape, orchards). Quantity of produced pollen and its nutritional value is currently the subject of bee-related studies. However, little is known about the unfavourable effects of heavy-metal contaminated pollen on bees. Moreover, the data on the quantity of heavy metals in pollen is still scarce.

We estimated pollen production in selected species (weeds, ornamentals) occurring in agricultural landscape of south-eastern Poland and collected pollen for heavy metal quantifications. Using ICP-OES method, we determined the quantities of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn.

For the pollen heavy metal contamination tests we chose 15 species that produced the largest amounts of pollen. In general, in the Asteraceae species pollen there were the highest quantities of heavy metals. In the focal species, Fe, Cu, Cr, Pb and V levels were elevated. The highest concentrations of Fe, V and Pb were found in *Solidago gigantea* and *S. canadensis* (mean = 1397-1390 µg/g, 2.3-2.8 µg/g and 3.7-3.8 µg/g, respectively). Pollen of *Hypericum perforatum* had the highest level (>2 µg/g) of Cd, while pollen of *Convolvulus arvensis* and *Linaria vulgaris* was most contaminated with Mn (mean = 103.1 and 98.9 µg/g).

The study species produced large amounts of pollen, however, usually they were contaminated with high concentrations of heavy metals. Further studies are necessary to explain the heavy metal presence and various concentration in pollen of different plant species.



### PP-169

#### Production of monofloral honey from Quillay (*Quillaja saponaria* Molina) as an ecosystem service of a native plantation generated by environmental compensation

Mauricio Rondanelli<sup>1</sup>, Iván Lamas<sup>1</sup>, Fabián Milla<sup>1</sup>, Eduardo Navarrete<sup>1</sup>, Pablo Novoa<sup>1</sup>, Javier Matamala<sup>1</sup>,  
María Carolina Iraira<sup>1</sup>, Lucía Vilches<sup>2</sup>

<sup>1</sup>Departamento de Ciencias y Tecnología Vegetal, Escuela de Ciencias y Tecnologías, Universidad de Concepción, Los Ángeles, Chile

<sup>2</sup>Gerencia de Medio Ambiente, Empresa Colbún, Santiago, Chile

Chile's forest ecosystems are generators of environmental services and non-timber forest products (NTFPs), among which beekeeping stands out as a complementary activity to those classically extractives from the forest. In the commune of Yumbel, Corderito farm sector, Biobío province, in central-southern Chile, the largest quillay plantation in the country is found, with 125.52 ha established in 2013 by the Chilean electricity producer Colbún, in the context of its environmental compensation activities. Given that the flowering of *Quillaja saponaria* favors the production of an emblematic honey in Chile and widely requested in foreign markets, the proposed research objective was to study the feasibility of producing monofloral quillay honey directing a melliferous production of local producers that favors the commercialization of a certified product of authenticity and origin. Experimental apiaries were installed in the land and honey was extracted from these once the flowering period of the species finished; The samples were analyzed by means of melissopalynological analysis to determine their botanical origin according to the procedure established in the Official Chilean Standard NCh2981-2005. The pollen percentage data for each determined taxon allow the construction of tables and percentage graphs of specific dominances. As a result, there is a record of 4 years (2020, 2021, 2022 and 2023) of analysis of the honey produced in the flowering season of the species (December-January), where for the seasons of 2020, 2022 and 2023 the honey harvested has been determined as quillay monofloral. For the 2021 season, the production of this honey was determined as multifloral, with a predominance of blackberry (*Rubus ulmifolius*) and strawberry tree (*Escallonia pulverulenta*) pollen, relegating quillay pollen to an approximate percentage of 5%. This coincides with an extremely dry year in the region, which would have had a negative impact on the flowering of *Q. saponaria*, favoring that of honey species with lower water requirements. The results of the research to date show the sensitivity of the phenologies of the floral resource to the climate and its consequence on honey production.

### PP-170

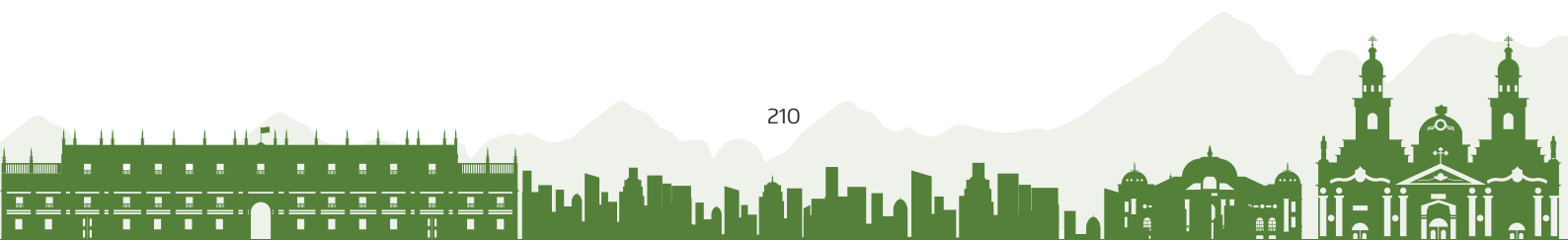
#### Novel strategy for melliferous native specie plant breeding program using nuclear techniques: radiosensitivity on Quillay

Daniel Alvaro Villegas Nassar<sup>1</sup>, Doris Ly<sup>1</sup>, Jorge Gonzalez<sup>2</sup>, Maria Paz Molina<sup>2</sup>, Patricio Vergara<sup>2</sup>

<sup>1</sup>Centro de tecnologías nucleares para ecosistemas vulnerables, Comisión chilena de energía nuclear.

<sup>2</sup>Instituto Forestal.

Beekeeping in Chile faces complex challenges due to the intensification of drought and increasing temperatures resulting from climate change. In this context, it is relevant to advance in the search for alternatives that allow our native melliferous species to adapt to these new conditions, for example through plant breeding programs. *Quillaja saponaria*, a relevant melliferous species, is one of the most affected by drought, with a significant decrease in the quantity and quality of its flowering, negatively impacting the national beekeeping industry. Recently, INFOR and CCHEN initiated studies to introduce ionizing radiation as a mutagenic agent in Quillay through the first studies of radio-sensitivity and determination of the lethal dose 50. Preliminary results indicate that Quillay seeds treated with increasing doses of gamma radiation (up to 200 Gy) show a decrease in parameters such as cumulative germination curve, final germination, germinative energy, and energy period. These results have allowed determining a mutagenic dose (LD50) close to 190 Gy, which is expected to be applied to selected lines of Quillay seeds in the search for generating genetic variability and resistance or tolerance to the drought and temperature increase conditions expected for the next decades.





### PP-171

#### Spatio-temporal availability of melliferous plant species for *Apis mellifera* in north-western Argentine Patagonia

Grecia Stefanía de Groot<sup>1</sup>, Suyai Svampa<sup>2</sup>, Marcelo Adrián Aizen<sup>3</sup>, Reto Schmucki<sup>4</sup>, Carolina Laura Morales<sup>1</sup>

<sup>1</sup>ECOPOL Grupo de Investigación en Ecología de la Polinización, Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA, CONICET-Universidad Nacional del Comahue), San Carlos de Bariloche, Argentina, Sociedad Latinoamericana de Investigación en Abejas (SOLATINA), Montevideo, Uruguay

<sup>2</sup>Universidad Nacional de Río Negro (UNRN), San Carlos de Bariloche, Argentina

<sup>3</sup>ECOPOL Grupo de Investigación en Ecología de la Polinización, Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA, CONICET-Universidad Nacional del Comahue), San Carlos de Bariloche, Argentina

<sup>4</sup>UK Centre for Ecology y Hydrology (UKCEH), Wallingford, UK

Beekeeping in north-western Argentine Patagonia is a small-scale production with great potential for development. Knowledge of the spatial and temporal distribution of the foraging plants that contribute to the nutrition of *Apis mellifera* hives is essential to plan and support such development. Classification of plant communities into units of melliferous flora can be a helpful tool for beekeeping and landscape management, informing beekeepers where and when the floral resources are available. We review the distribution and the flowering time of melliferous species found in plant communities growing in north-western Argentine Patagonia. We then characterize the richness and composition of these communities and we identify melliferous flora units (MFU) and their flowering curves (phenology) based on their similarity. We identified a total of 72 botanical families, 191 genera and 254 melliferous species (114 native and 140 exotic). Melliferous species were distributed across 20 plant communities grouped in nine MFU, with the highest richness in anthropic and arid units. In general, the greatest richness of flowering species occurs between November and January, with a peak of nearly 170 species blooming between December and January. These results highlight the value -in terms of diverse melliferous floral resources- of anthropic and arid units. Thus, targeting beekeeping to these environments may benefit honeybee nutrition and at the same time, reduce the pressure on natural environments and native pollinators. The richness of melliferous plant species in the early summer highlights the need to properly prepare hives to benefit from this diverse flow of nectar. However, the high number of exotic plant species used by *Apis mellifera* highlights the importance of carefully plan and monitor the development of beekeeping, recording the location of apiaries and number of hives, as well as the effects on native pollinators and the reproductive rates of melliferous plant species.

### PP-172

#### Characteristics of squash (*Cucurbita maxima*) fruits pollinated with honey bees and bumblebees (*Bombus pauloensis*)

Sheena Salvarrey<sup>1</sup>, Yulai Ramos<sup>1</sup>, Fernanda Zaccari<sup>1</sup>, Luis Barboza<sup>1</sup>, Leandro Arrillaga<sup>2</sup>, Olga Pascual<sup>2</sup>, Diego Romero<sup>2</sup>, Ciro Invernizzi<sup>1</sup>

<sup>1</sup>Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

<sup>2</sup>Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay.

In Uruguay, the production of squash (*Cucurbita maxima*) under greenhouses out of season has had a sustained growth favored by high prices. This crop is highly dependent on entomophilous pollination (90%), so it is usually accompanied by pollination by honey bees, despite the difficulties they have to work in greenhouses. The use of bumblebees, recognized for their good performance in greenhouses, could contribute more efficiently to fruit production. This work aims to evaluate the effect of pollination by honey bees and native bumblebees, *Bombus pauloensis*, on squash fruits under greenhouse conditions. To do this, four bumblebee colonies were installed in a squash crop and one *A. mellifera* colony under an 800 m<sup>2</sup> greenhouse. For this, flowers were closed and left exposed, presenting 4 groups: 1 visit by *A. mellifera*, free pollination by *A. mellifera*, 1 visit by *B. pauloensis* and free pollination by *B. pauloensis*. Once the fruits of squash were formed from the marked flowers, they were harvested and processed in the laboratory where different fruit quality indicators were determined. The results showed that the fruits obtained from flowers exposed to free pollination with bumblebees appeared higher in: weight per fruit, fruit height and diameter of the seminal cavity (Tukey Test,  $P < 0.05$ ). In the other characteristics evaluated, such as: number of seeds, equatorial diameter and height of the fruit, no differences were found between the different groups evaluated. On the other hand, the group of flowers that received a visit or free pollination by bumblebees did not give rise to deformed fruits, while the groups pollinated by *A. mellifera* did. This study shows that the use of bumblebees as squash pollinators in greenhouses can improve the yield and quality of the fruits obtained.



### PP-173

#### Systematic evaluation of the polliniferous flora in the cliserie forest linked to a productive beekeeping system in Belen and Cerinza (Boyacá Colombia)

Guillermo Salamanca Grosso<sup>1</sup>, Freddy Alexander Borja Peralta<sup>2</sup>, Monica Patricia Osorio Tangarife<sup>3</sup>

<sup>1</sup>Departamento de Química Facultad de Ciencias Universidad del Tolima

<sup>2</sup>Grupo de Investigaciones Mellitopalínológicas y Propiedades Físicoquímica de Alimentos Facultad de Ciencias Universidad del Tolima

<sup>3</sup>Laboratorio de Propiedades Físicoquímica de Alimentos Facultad de Ciencias Universidad del Tolima

The determination of the species of beekeeping interest that predominate in beekeeping environments contributes significantly to the development of productive beekeeping systems. We have evaluated and systematized the pollen flora of the beekeeping environment in the areas of influence of Belén and Ceriza (Boyacá) and established their rhythms considering aspects of floral phenology.

*Apis mellifera* uses a large number of resources as a source of pollen and nectar. For the study area 106 botanical species were related, distributed in 42 families, 82 species for the zoa and 45 for Cerinza. The work has made it possible to relate 1 The bee flora is distributed in six main groups, with a percentage predominance in Compositae 27, Ericaceae 12, Melastomataceae

7, Leguminosae, Rosaceae and Verbenaceae. In relation to the predominant specimens, *Baccharis* sp, *Taraxacum campyloides*, *Bidens* sp. (Compositae), *Weinmannia tomentosa*, *Cunnoniaceae*, *Cunnoniaceae* and *Cunnoniaceae. tomentosa* (Cunnoniaceae), *Miconia* sp. and *Monochaetum* sp. (Melastomataceae), *Dodonea viscosa*, (Sapindaceae), *Trifolium repens* (Leguminosae), *Morella parvifolia*, (Myricaceae), *Vallea stipularis* (Elaeocarpaceae), *Myrcianthes leucoxylla* (Myrtaceae), *Duranta mutisii* (Verbenaceae), *Bejaria resinosa*, *Macleania rupestris*, *Gaultheria* sp, *Pernettya* sp (Ericaceae) mainly.

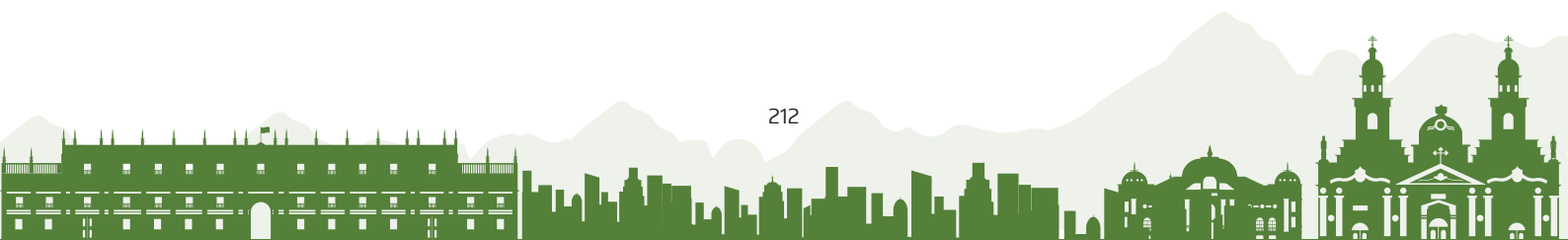
### PP-174

#### BeeNet: monitoring bee networks to evaluate the Italian agroecosystem

Giovanni Cilia, Sergio Albertazzi, Gherardo Bogo, Michela Boi, Vittorio Capano, Elena Cargnus, Emanuele Carpana, Francesca Corvucci, Amanda Dettori, Simone Flaminio, Manuela Giovanetti, Irene Guerra, Giulia Lora, Piotr Medrzycki, Antonio Nanetti, Marino Quaranta, Giorgia Serra, Elena Tafi, Laura Zavatta, Laura Bortolotti

CREA Research Centre for Agriculture and Environment, Bologna, Italy

Monitoring the health of the agricultural environment is an increasingly relevant issue within the European Union's agriculture and rural development policies. In particular, there is an emerging need for tools to monitor the effectiveness of agri-environmental measures put in place by the EU for rural areas. The BeeNet project, funded by the Italian National Rural Network, aims to monitor the health of the Italian agroecosystem through two monitoring networks: honey bee colonies and the wild bee biodiversity. The first network is composed of 363 sites with 1815 beehives, distributed across the whole national territory. This monitoring consists in evaluating the status of the investigated colonies, in terms of growth and development during the year, and through the presence of pathogens in honey bee adults and pesticide contamination in beebread. The second network evaluates the number and diversity of wild bees in 26 fixed transects located in 11 regions and positioned in two different agro-environments, one intensively cultivated and one semi-natural. The collected specimens, identified at the species level, are related to the blooms present on the day of sampling. Concerning the pathological data, the analyses highlighted the ubiquitous presence in Italy of *Nosema ceranae* and the viruses DWV, and CBPV. Also, lower prevalence was found for ABPV and KBV and ABPV. Among trypanosomatids, *Lotmaria passim* was only sporadically detected in the honeybee samples, while *Crithidia mellifica* and *Crithidia bombi* have never been found. The pesticide residue analysis in beebread samples showed as two-thirds of the investigated samples contain one or more active compounds. Besides, many contain between two and five residues, while some had over ten residues. In addition, almost a third of the positive samples contain at least one active compound highly toxic to bees and 20% contain only active ingredients with low toxicity for bees. The results of the wild bee biodiversity network highlighted that semi-natural agroecosystems have higher biodiversity (both in terms of species richness and individual abundance) of wild bees, compared to intensive agroecosystems. BeeNet project ensures a constant commitment to secure scientific data gathering and to collaborate with various stakeholders.



## Pollination and Bee Flora

### PP-175

### Public innovation for the development of a safe pollination service in view of the growing fruit area in the Los Lagos Region – Chile

Nolberto Arismendi<sup>1</sup>, Nelson Caro<sup>2</sup>, Marcelo Vera<sup>3</sup>, Erwin Guzman<sup>1</sup>

<sup>1</sup>Centro de Investigación Austral Biotech, Facultad de Ciencias, Universidad Santo Tomás. Av. Ramón Picarte 1130-1160, Valdivia

<sup>2</sup>Centro de Investigación Austral Biotech, Facultad de Ciencias, Universidad Santo Tomás. Ejército Libertador 146, Santiago

<sup>3</sup>Oficina de Proyectos, Universidad Santo Tomás. Av. Ramón Picarte 1130-1160, Valdivia

In Chile, there is less water availability in several fruit-growing regions in the north of the country, which has caused fruit orchards to move to the south of the country in search of the best water and climatic conditions for their production. Within this context, the Los Lagos region has significant potential for the development of Chilean fruit growing, which generates an opportunity for pollination services based on bees, but also a risk due to being exposed to the application of pesticides, especially in critical flowering periods in spring. In this context, the question arises, how can we pollinate safely in the face of the growing fruit area in the Los Lagos region without having an adverse effect on bees? For this, it has been proposed to co-design and implement with producers, beekeepers and other relevant actors, innovative solutions that contribute to developing safe pollination in the face of the growing fruit area in the Los Lagos region. Thus, based on the Double Diamond model, we intended to identify, define and provide a solution to the identified problem. Preliminary results in working groups with relevant regional stakeholders, 54 current and potential problems were identified, of which 29% of the problems identified were considered serious or very serious, which must be resolved almost in their entirety (84%) in the short or medium term. Thus, problems grouped in the application of agrochemicals and their implications, professionalization of the pollination service, territorial planning associated with beekeeping, training of beekeepers and/or fruit growers, among others, were the most relevant problems identified. In a second stage of work, it is expected to co-design a solution that allows solving most of the identified problems, to later develop a prototype, develop activities of a pilot, implement and transfer the developed solution to the relevant actors associated with a safe pollination activity in the Los Lagos Region, which will strengthen the relationship between beekeepers and fruit growers based on open innovation that involves components from the public, private and academia sectors.

### PP-176

### Using nuclear techniques to evaluate climatic changes on agriculture management and in hence honeybee production

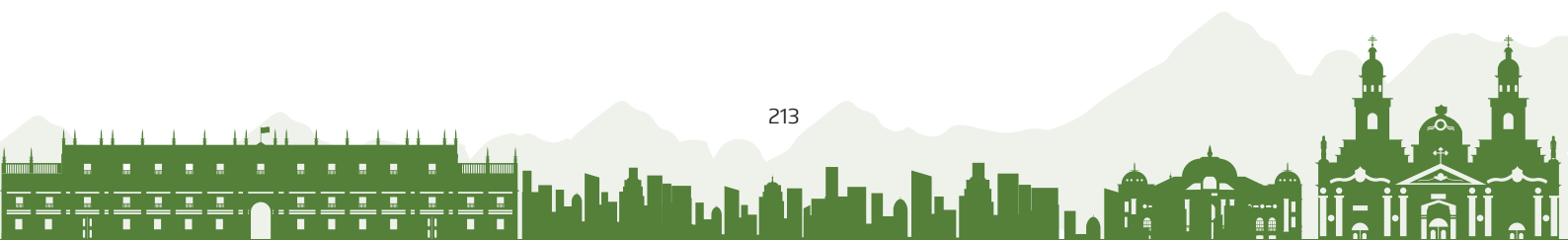
Adriana Nario

Nuclear Technologies Centre in Vulnerable Ecosystems (NTCVE), Chilean Nuclear Energy Commission, Santiago, Chile

One of the major and seriousness problem that affects the agriculture production in Chile and thus, pollinators activities such as melliferous bees, its related with the loss of soil's productive capacity caused by the severe erosion process, mainly because of an intensive and unsuitable management, affecting 60% of the agriculture productive area of Chile. Other problem associated with the agriculture activity is the impact of climatic changes as water scarcity and high temperatures, influencing the presence of different pests and in hence the use of pesticides affecting honey quality and yields.

The use of nuclear techniques is essential to acquaintance the impact of the anthropic factors (pesticides, fertilization, and agriculture management) over the biotics (microorganisms) and abiotics (soil and water) elements to sustain an environmental and productive management and maintain or increase healthy bees and honey production. The results and the management model developed will be in direct benefits to the farmers and will be applied in the governments programs (PRSD and PMP), under the Regional Services supervision with the support of other publics institutions and agriculture management tools.

The study has the following OBJECTIVES: (1) Introduce and use of isotopic techniques to study the agriculture management process, (2) evaluate the agriculture management impact on the honey net production and the relation with their properties and (3) create a database for the develop of the catchment management model.



### PP-177

### Povi Poeh ~ The Flower Path ~ Puebloan Indigenous Matriarchs inspiring pollinator habitat regeneration in climate change fueled wildfire impacted landscapes along the northern Rio Grande & Rocky Mountains

Melanie Kirby<sup>1</sup>, Roxanne Swentzell<sup>2</sup>, Teresa Kuality Quintana<sup>3</sup>, Addelina Lucero<sup>4</sup>, Beata Tsosie Pena<sup>5</sup>

<sup>1</sup>Nectar Nomad Productions, Zia Queenbees Farm & Field Institute, Institute of American Indian Arts

<sup>2</sup>Flowering Tree Permaculture Institute

<sup>3</sup>Institute of American Indian Arts

<sup>4</sup>Dancing Butterfly Naturals

<sup>5</sup>Espanola Healing Food Oasis

Povi Poeh ~ The Flower Path ~ is a collective of Puebloan Indigenous Matriarchs from Tiwa, Tewa, and Keres speaking tribal communities along the northern Rio Grande and southern Rocky Mountain region working together to help educate and encourage the regeneration of pollinator habitat. In the summer of 2022, the state of New Mexico in the southwestern United States, experienced the largest wildfire in its history due to the merging of man-caused and federally ignited uncontrollable wildfires in the Sangre de Cristo Mountain range. In the summer of 2022, the Hermit's Peak and Calf-Canyon fires burned for 81 days, and spanned an area of close to 500,000 square feet (46500 square meters) spanning over 51 miles long (82 kilometers). These merged fires caused catastrophic loss of forest, conifer and high elevation trees, grasses, alpine wildflowers, and critical wildlife habitat. Following the fires was heavy summer monsoon rains which caused severe flooding and washing away of soot, ash, and burnt vegetative debris downstream into rangelands and pastoral Indo-Hispano mountain communities. The Povi Poeh collective of Puebloan Indigenous Matriarchs includes Indigenous food sovereignty scholars, artists, farmers, and beekeepers working together to develop creative outreach materials and community gathers for regenerating these damaged forest and surrounding high desert landscapes and communities. Efforts include Indigenous methodologies of holistic biodynamic stewardship, conscientious permaculture, and agroecological applications. The collective is developing culturally relevant field guides through an Indigenous lens based in ecosystem interconnectedness between people, pollinators, plants for nurturing youth to elder calls to action for regenerating pollinator habitat and biodiversity conservation in climate-change impacted landscapes.

### PP-178

### Optimization of bee-collected pollen DNA extraction for ITS2 metabarcoding

M. Alice Pinto<sup>1</sup>, Andreia Quaresma<sup>1</sup>, Robert Brodschneider<sup>2</sup>, Norman Carreck<sup>3</sup>, Kristina Gratzner<sup>2</sup>, Fani Hatjina<sup>4</sup>, Ole Kilpinen<sup>5</sup>, José Rufino<sup>6</sup>, Flemming Vejsnæs<sup>5</sup>, Jozef Van Der Steen<sup>7</sup>, Alexander Keller<sup>8</sup>

<sup>1</sup>CIMO, SUSTEC, Instituto Politécnico de Bragança, Portugal

<sup>2</sup>Institute of Biology, University of Graz, Austria

<sup>3</sup>Carreck Consultancy Limited, UK

<sup>4</sup>Ellinikos Georgikos Organismos DIMITRA, Greece

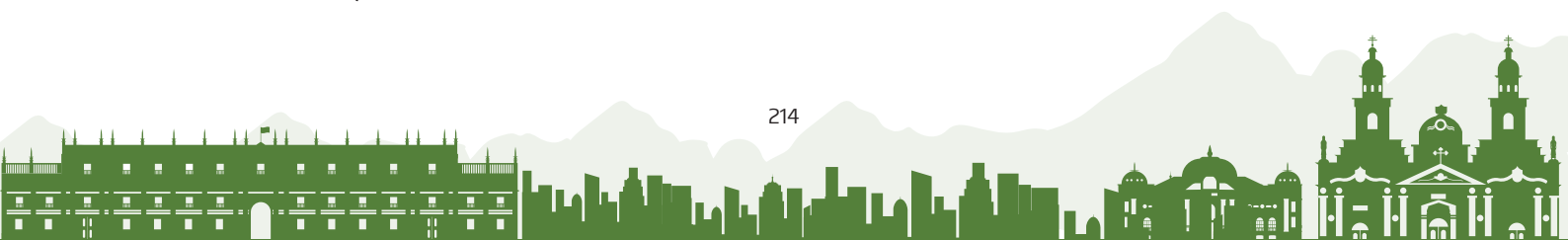
<sup>5</sup>Danish Beekeepers Association, Denmark

<sup>6</sup>CeDRI, SUSTEC, Instituto Politécnico de Bragança, Portugal

<sup>7</sup>Alveus AB Consultancy, Netherlands

<sup>8</sup>Faculty of Biology, Ludwig-Maximilians-Universität Munich, Germany

The quality of the environment can be studied by analysing the pollen collected by honey bees, as it contains information on available plant sources, as well as spatial and temporal floral diversity. This requires the botanical identification of bee-collected pollen, which has typically been addressed by classical palynology, a time-consuming approach. However, with high-throughput sequencing becoming increasingly affordable, pollen metabarcoding is thriving, and is a promising alternative to classical palynology, especially for projects involving large numbers of samples. A bottleneck in the metabarcoding laboratory pipeline for large projects is DNA extraction. The scientific literature reports on DNA extraction using commercial kits, which involve manual processing. However, for large numbers of samples, manual extraction is a time-consuming, tedious, and prone to cross-contamination endeavour. The INSIGNIA-EU citizen science project involves the analysis of over 3,000 pollen samples collected across Europe, requiring an alternative to manual extraction. Herein, we tested an automatic DNA extraction protocol on 41 pollen samples collected in Austria, Denmark, France, Greece and the UK. We compared the relative abundances obtained from high-throughput metabarcoding of ITS2 between both DNA extraction protocols. We extracted DNA from the 41 pollen homogenates with one of the most popular manual commercial kits (Macherey-Nagel NucleoSpin Food Kit) and with the Maxwell® (Promega) automatic extractor using the Maxwell® RSC PureFood GMO and Authentication Kit. The two methods produced similar results. The manual extraction detected 112 families and 50 genera, and the automatic extraction detected 113 families and 49 genera. The two protocols detected the same top-5 families with very close relative abundances (Brassicaceae: 13.8% and 14.0%; Fabaceae: 13.1% and 14.2%; Asteraceae: 12.4% and 11.5%; Rosaceae: 10.8% and 12.8%; Plantaginaceae: 10.8% and 9.0%, for automatic and manual extractions, respectively). The Shannon diversity index did not differ (p-value = 0.479, Kruskal-Wallis test) between the two extraction protocols (median: family=1.5 and 1.6 for automatic and manual extractions, respectively; genus=1.7 for both protocols). Our results indicate that the automatic extraction can confidently replace the standard manual extraction method, allowing easy scaling up of pollen analysis (1 hour for extracting 48 samples as compared to ~5 hours to extract 24 samples).





### PP-179

#### Conservation approach of "Farming with Alternative Pollinators": success and drivers

Ahlam Sentil<sup>1</sup>, Patrick Lhomme<sup>2</sup>, Sara Reverté<sup>1</sup>, Insafe El Abdouni<sup>2</sup>, Laila Hamroud<sup>2</sup>, Orianne Rollin<sup>3</sup>, Oumayma Ihsane<sup>2</sup>, Youssef Bencharki<sup>2</sup>, Pierre Rasmont<sup>1</sup>, Moulay Chrif Smaili<sup>4</sup>, Denis Michez<sup>1</sup>, Axel Ssymank<sup>5</sup>, Stefanie Christmann<sup>2</sup>

<sup>1</sup>Department of Zootechnics, Federal University of São Francisco Valley, Petrolina, Pernambuco, Brazil

<sup>2</sup>Department of Zootechnics, Paulista State University "Júlio de Mesquita Filho", Botucatu, São Paulo, Brazil

<sup>3</sup>Collegiate Graduate Program in Ecology and Evolution, Feira de Santana State University, Feira de Santana, Bahia, Brazil

The species "*Passiflora foetida* L." (Passifloraceae) is distributed in all regions of Brazil, and has as its main botanical characteristics hermaphrodite flowers with the production of nectar as its main resource provided to pollinators, which are predominantly bees. The objective of the study was to verify the influence of the main pollinating bees of "*P. foetida*" on the reproductive success of the species. The study was conducted with six natural populations of "*P. foetida*" located in an area of the Caatinga biome (Petrolina, Pernambuco State, Brazil). Floral visiting bees were counted for ten non-consecutive days during flower anthesis at each one-hour interval (between 5 and 11 am), being considered potential pollinators those bees that came in contact with the reproductive structures of "*P. foetida*" during floral visits. To verify the efficiency in pollination by bees, flower buds in pre-anthesis were protected with voil tissue and used in the four treatments performed: spontaneous self-pollination (n = 30; flowers remained protected throughout anthesis); manual self-pollination (n = 30; flowers pollinated with pollen from the same individual); manual cross-pollination (n = 30; flowers pollinated with pollen from different individuals), and control (n = 60; flowers free for bees to visit). After fertilization of the flowers, the number of fruits formed for each treatment was counted, and the fruit set rate was calculated (number of fruits formed x 100 / number of flowers marked). Of the nine bee species that visited "*P. foetida*", only "*Apis mellifera*", "*Melipona mandacaiá*" and "*Peponapis fervens*" were considered potential pollinators, based on the frequency of visits, morphology and intrafloral behavior, representing 57.27% of the total visits by bees in "*P. foetida*". With the exception of the spontaneous self-pollination treatment, in the other treatments there was fruit production. The highest production rate was recorded in the manual cross-pollination treatment (73.33%), followed by the control treatment and manual self-pollination (41.67 and 16.67%, respectively). This study shows the importance of bee diversity for pollination of "*P. foetida*", and biotic agents such as bees are needed for greater efficiency in pollination of this passion fruit.

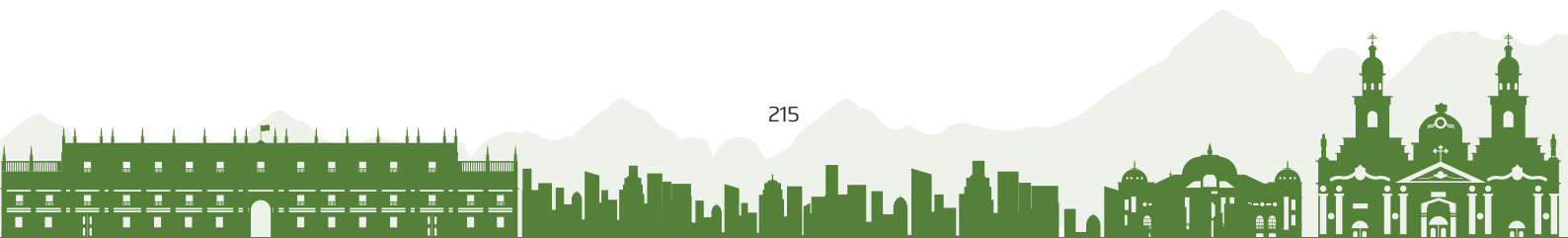
### PP-180

#### "The Salars of northern Chile, vulnerable surrounding ecosystems and impact on sustainable beekeeping"

Ana Valdés, Enrique Mejías

Centro de Tecnologías Nucleares en Ecosistemas Vulnerables, División de Investigación y Aplicaciones Nucleares, Comisión Chilena de Energía Nuclear

Northern Chile presents from north to south basins occupied by saline lakes and salt crusts, known as salars (Risacher et al., 2003). There are more than 50 of salars, distributed in 7 main morphostructural units that from west to east are: 1) the Coast Range, 2) the Central Depression, 3) the Precordillera, 4) the Pre-Andean Depression, 5) the Western Cordillera, 6) the Altiplano 7) the Eastern Cordillera (Risacher et al., 2003). The three predominant types of salt flats correspond to a mixture between Playas, Saline Lakes and Salt Crusts, active and fossil (Risacher et al., 2003). The Salar de Atacama is located between the Cordillera de Domeyko (Pre-Cordillera), to the west, and the West Cordillera, to the east (Álvarez et al., 2022). Some described environmental characteristics of these ecosystems are associated with the lower areas of the Salar de Atacama, where groundwater emerges, characterized by high solar radiation, extreme daily temperature fluctuations typical of desert environments, net evaporation that produces hypersaline water and, high arsenic content in the water due to volcanic events (Farías et al., 2014). These conditions contribute to an environment that selects for microbial extremophiles (Farías et al., 2014). The hypersalinity and hyperdensities of the waters impact the interaction between saline and fresh waters, consequently, in the preservation of adjacent environments, such as wetlands, among others. San Pedro de Atacama is located 55 km north of the Salar de Atacama. The Vasquez Study. 2010, botanically determined four types of honey in San Pedro de Atacama: *Prosopis alba*, *Geoffroea decorticans*, *Prosopis* sp. and polyfloral, presenting differences in their values for the physical and chemical parameters. Of the pollens analyzed, the majority correspond to species of native origin and a smaller proportion to introduced species. Of the samples analyzed, 74% are monofloral honeys and 26% polyfloral. The biogeochemical profile of bee products differs from other honeys produced in the same region, in locations far from salt flats. The characterization of the origin of honeys and pollens carried out with biogeochemical parameters could be a complementary tool at the service of the authenticity certification of Chilean bee products.





### PP-181

### Bees pollinate "*passiflora foetida*" (passifloraceae) in the brazilian semiarid

Catiana Da Conceição Vieira Melquiades<sup>1</sup>, Yan Souza Lima<sup>2</sup>, Francisco Israel Lopes Sousa<sup>1</sup>, Francisco De Assis Ribeiro Santos<sup>3</sup>, Eva Monica Sarmento Da Silva<sup>1</sup>

<sup>1</sup>University of MONS, Research institute for Biosciences, Laboratory of Zoology, 7000 Mons, Belgium

<sup>2</sup>International Center of Agricultural Research in the Dry Areas, Station Exp. INRA-Quich, 10112 Rabat, Morocco

<sup>3</sup>CARI, Beekeeping center of research and information, 1348 Louvain-la-Neuve, Belgium

<sup>4</sup>National Institute of Agricultural Research, Regional Center of Agricultural Research of Kenitra, Laboratory of Entomology, 14000 Kenitra, Morocco

<sup>5</sup>German Federal Agency for Nature Conservation (Bundesamt für Naturschutz, BfN), 53179 Bonn, Germany

Global reliance on pollinator dependent crops increased very steeply, while evidence on pollinator decline has been reported in all continents. Globally affordable conservation strategies need to be developed, as high-cost measures like European agri-environment schemes are not scalable in all countries. Here, we test, if a low-cost conservation approach named "Farming with Alternative Pollinators" (FAP) can benefit flower visitor abundance and richness in agro-ecosystems and in crops, through establishment of Marketable Habitat Enhancement Plants (MHEP). The study was carried out in four Moroccan agro-climatic regions, during two years, using 6 main crops (pumpkin, zucchini, faba bean, tomato, eggplant and apple) and 201 fields. Additionally, we investigated how crop type, crop-MHEP composition (i.e. phylogenetic distances among crop and MHEP) and local climate can drive the success of the approach in comparison to monocultural fields. Based on 7097 recorded specimens, our results show that the flower visitors of the entire FAP fields (i.e. 75% main crop and 25% MHEP) were significantly more abundant and species-rich than those of control fields (i.e. 100% main crop). Considering the flower visitors of the main crop, FAP increased their abundance, while a neutral effect of the approach on their richness was reported. The mean phylogenetic distance between the main crop and MHEP, and climatic variables were not correlated with increase in flower visitor abundance and richness in FAP fields. The crop type was found to influence the effect of the FAP approach. Our study provides strong evidence that FAP constitutes a relevant method for flower visitor conservation in agro-ecosystems and a potential tool to promote crop pollination service. Further research on additional environmental factors is necessary to outline the circumstances under which the FAP approach can positively affect flower visitor communities.

### PP-182

### Bee frequency in flowers of the clock plant "*Sida acuta* var. *carpinifolia*", malvaceae in a caatinga area

Eva Silva<sup>1</sup>, Mirelly Alexandre Gomes<sup>2</sup>, Maria José Sarmento Filha<sup>3</sup>, Gerlania Sarmento Verissimo<sup>4</sup>, Yan Souza Lima<sup>5</sup>, Catiana Da Conceição Vieira Melquiades<sup>1</sup>, Francisco Israel Lopes Sousa<sup>1</sup>, Tania Maria Sarmento Da Silva<sup>6</sup>

<sup>1</sup>Collegiate of Zootechnics, Federal University of the São Francisco Valley, Petrolina, Brazil.

<sup>2</sup>Department of Fundamental Chemistry Federal University of Pernambuco, Recife, Brazil

<sup>3</sup>City Hall of Vieirópolis, Paraíba, Brazil

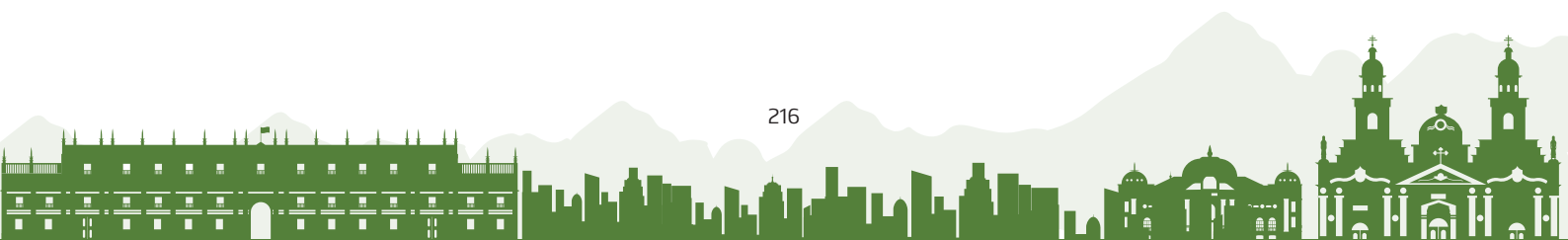
<sup>4</sup>University Hospital Lauro Wanderley, João Pessoa, Brazil

<sup>5</sup>Paulista State University Julio de Mesquita Filho, São Paulo, Brasil

<sup>6</sup>Departament of Chemistry Federal Rural University of Pernambuco, Recife, Brazil

The species "*Sida acuta* var. *carpinifolia*" (L.f.) Schum. is a subshrub of the Malvaceae family that has been studied for its ethnomedicinal properties and as bee flora. Due to its occurrence in several Brazilian regions, especially the semi-arid region, it is important to know its relationship with the bee fauna that occur in this region. The objective of this study was to verify the frequency of flower visits by bees in "*S. acuta*" in a caatinga area. During five consecutive days, the floral visiting bees were recorded during the floral anthesis, in "*S. acuta*" populations located in a Caatinga area (Sousa, Paraíba State, Brazil). Floral visits were monitored focally, and the number of visiting bees was counted for 15 minutes at each one-hour interval. ANOVA was performed to analyze the flow of visits, and for comparison of means, Tukey's test was used at 5% significance level. The flowers of "*S. acuta*" have a diurnal anthesis period (between 07 and 11 hours), providing pollen and nectar as floral resources for their visitors. A total of 235 total floral visits were recorded during the study, performed by the bee species "*Apis mellifera*"(54.47%) and "*Augochlora*" spp.(45.53%). Regarding the frequency of visits, "*Apis mellifera*" had the peak of visits at 09:00 am ( $F = 4.5$ ;  $GL = 4$ ;  $p = 0.01$ ), while species of the genus "*Augochlora*" showed no significant differences in relation to the times of visits ( $p = 0.6$ ). Although "*S. acuta*" has a short anthesis period, a significant number of floral visits were made by bees, and it can be considered an important source of floral resources for wild and honey bees in areas of the Caatinga biome.

Acknowledgment: CNPq, CAPES, FACEPE



### PP-183

#### National public policy for pollinator and pollination conservation in Brazil: a regional analysis

Jenifer Dias Ramos<sup>1</sup>, Patrícia Nunes-Silva<sup>2</sup>, Carlos Demeterco<sup>3</sup>

<sup>1</sup>Laboratório de Entomologia e Fitopatologia, Embrapa Meio Ambiente, Jaguariúna/São Paulo, Brazil

<sup>2</sup>Escola Politécnica, Unisinos, São Leopoldo, Brazil

<sup>3</sup>INPA

The conservation of pollinators and pollination relies on the existence of public policy. Here we analyzed the main legal frameworks for pollinator conservation in Brazil, at federal and state levels, to point out the current gaps and to recommend actions to promote effective conservation, increase and maintenance of pollinator populations in agroecosystems, and, consequently, pollination services. For this, we surveyed public policy that are directly and indirectly linked to the conservation of pollinators and pollination services: beekeeping, pesticide use, creation of conservation areas, incentives to organic farming, awareness and economic incentives. The search was done on the official government websites and on scientific literature that had previously compiled this information. Brazilian legislation (federal and state) related to the ecosystem service of pollination, conservation of pollinators and beekeeping, could be divided into thematic areas: (a) pesticides and their state consequences stood out; (b) the regulation of meliponiculture, which is still a gap in some spheres; (c) the regulation of beekeeping and honey production; (d) regulations and encouragement of agriculture and organic production; (e) protection and action plans for biodiversity and conservation. 45 federal laws and 187 state laws were related to pollination and pollinators. Only six Brazilian states (of 26) have 10 or more laws on the topics: Rio Grande do Sul (19; South), Pernambuco (18; Northeast), Rio de Janeiro (14; Southeast), Piauí (13; Northeast), São Paulo (11; Southeast) and Bahia (10; Northeast). At the national level there are four policies related to the conservation of pollinators, encouraging the agroecological transition and reducing the use of pesticides, factors that contribute to the protection of pollinators: National Policy on Agroecology and Organic Production (PNAPO), National Program for the Strengthening of Family Agriculture (Pronaf), National Environment Policy (PNMA) - LAW No. 6,938/81 and National Policy of Biosafety - PNB. From these data, it was possible to identify the need to expand municipal and state actions to conserve pollinators and the landscape. In addition to the expansion of federal public policies aligned with the 2030 agenda and the sustainable development objectives, where pollinators came to have a prominent place.

### PP-184

#### Pollen diversity stored by *Trigona spinipes* in the Brazilian semiarid region

Catiana Da Conceição Vieira Melquiades<sup>1</sup>, Yan Souza Lima<sup>2</sup>, Francisco Israel Lopes Sousa<sup>1</sup>, Juliana Duarte De Brito Campos<sup>1</sup>, Lucas Moura Ribeiro Afonso<sup>1</sup>, João Henrique Alves Da Cruz Coelho<sup>1</sup>, Marileide Souza Sá<sup>1</sup>, Lucas Eugenio Pereira Barbosa<sup>1</sup>, Francisco De Assis Ribeiro Santos<sup>3</sup>, Tania Maria Sarmento Silva<sup>4</sup>, Eva Monica Sarmento Silva<sup>1</sup>

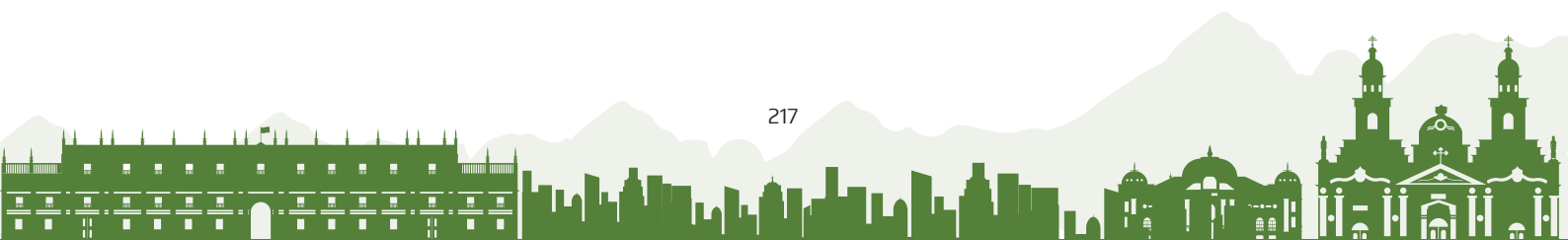
<sup>1</sup>Department of Zootechnics, Federal University of São Francisco Valley, Petrolina, Pernambuco, Brazil

<sup>2</sup>Department of Zootechnics, Paulista State University "Júlio de Mesquita Filho", Botucatu, São Paulo, Brazil

<sup>3</sup>Graduate Collegiate in Ecology and Evolution, State University of Feira de Santana, Feira de Santana, Bahia, Brazil

<sup>4</sup>Department of Chemistry, Federal Rural University of Pernambuco, Recife, Pernambuco, Brazil

The species *Trigona spinipes*, known as "arapuá", is one of the representatives of the stingless bee biodiversity present in Brazil; being known for its generalist habit in relation to the floral sources visited. Considering its pollination potential, it is important to identify botanical sources associated with the pollen diversity collected by *T. spinipes*. The study was conducted in a Caatinga area (Petrolina, Pernambuco State, Brazil), where pollen samples were collected from hives of arapuá bees monthly, during 12 months. The collected pollen material (pollen grains) was acetolyzed and the pollen types were identified based on the local palynoteca and specialized literature. The diversity and distribution frequency of pollen types in relation to the months sampled was calculated, followed by the Shannon-Weaver diversity index ( $H'$ ) and Pielou's equalibility index ( $J'$ ). A total of 31 pollen types were recorded in the pollen samples stored by *T. spinipes*, with Fabaceae and Malvaceae being the main botanical families represented ( $n = 6$  and  $3$  pollen types, respectively). The pollen types with the highest distribution frequency are associated mainly with species endemic to the Caatinga biome, especially the species *Mimosa tenuiflora* (Fabaceae), present in all months analyzed. During the study, we observed higher pollen diversity ( $H' = 2.44$  and  $J' = 0.88$ ) related to the higher rainfall rates in the region, probably associated with the abundance of flora available in the area. *T. spinipes* has a wide spectrum of collection of floral resources for pollen supply, with a predominance of pollen types associated with endemic species of the Caatinga biome. Acknowledgment: CNPq, CAPES, FACEPE.



### PP-185

#### ¿Are solitary bees effective pollinators for crops in Chile?

Victor Hugo Monzon, José Mesquita-Neto, Ruben Garrido

Laboratorio de Ecología de Abejas. Facultad de Ciencias Básicas. Universidad Católica del Maule. Talca-Chile

Pollination is a fundamental function for ecosystems allowing the reproduction of native flora and agricultural crops. *Apis mellifera* is currently the most often utilized bee in pollination services, largely because of its all-purpose foraging activity and convenient handling. Other types of bees, however, also contribute significantly to the pollination of numerous plant species. These solitary bees perform ecosystem services such as pollination in agroecosystems, and in some situations, they are even more efficient pollinators than honey bees. In this regard, a greater diversity of pollinators may enhance the quantity and quality of pollination due to a synergistic effect known as complementary foraging, which would result in a higher yield of fruits of higher quality. It is important to conduct further research in this area because the utilization of these bees has not received much attention in our country. The goal of the current study is to assess the pollination function of Chile's solitary wild bees, which helps to increase output in the agro-fruit industry, particularly in the avocado, blueberry, and sour cherry crops grown in Central Chilean regions. According to the data, a wide variety of solitary bee species exist, including, among others, *Colletes seminitidus*, *Caupolicana gayi*, *Cadeguala occidentalis*, *Corynura chloris*, and *Corynura chilensis*. These bees typically visit fewer flowers per minute than *A. mellifera*, but they linger in each bloom for a considerably longer period of time, which may contribute to their superior pollination effectiveness.

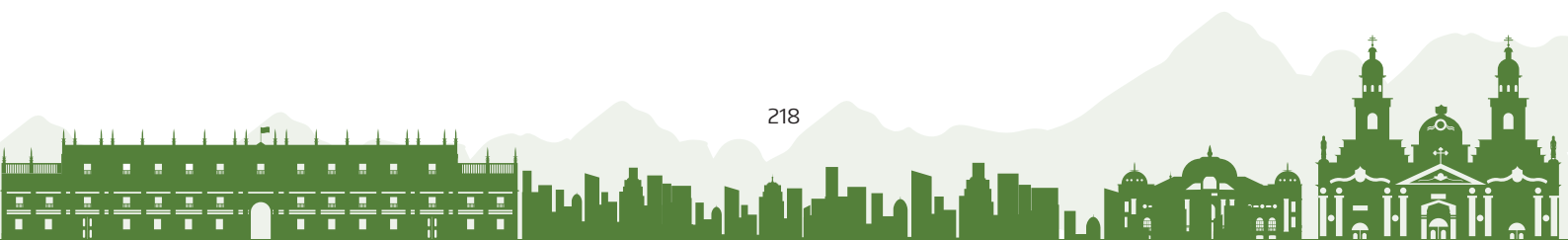
### PP-186

#### Morphology of pollen grains of the predominant vegetable species in the populated center of Agocucho, Cajamarca, Perú

Nelly Maribel Vásquez Bustamante, Alonso Vela Ahumada, Jhon Anthony Anthony Vergara Copacondori

Department of Agronomy, National University of Cajamarca, Cajamarca, Perú

In seven hamlets of the Agocucho Populated Center, district, province and department of Cajamarca, Peru. The predominant bee flora was collected to characterize morphologically its pollen grains. During six months, its vegetative structures (stem, leaf, flower and fruit) were taxonomically identified to build an herbarium of reference. "The pollen, for its characterization, was obtained from flowers and from beekeepers' traps installed in bee hives *Apis mellifera*". Later, it was observed in the laboratory through an electronic microscope to obtain a palinotheque. Thirty-one species were taxonomically identified, among them Asparagaceae, Malvaceae, Cactaceae, Brassicaceae, Poaceae, Solanaceae, Cucurbitaceae, Verbenaceae, Fabaceae, Myrtaceae, Anacardiaceae, Rosaceae, Rutaceae y Asteraceae. "Through a palynological analysis, the presence of 45 plant species, distributed in 20 botanical families, was determined, A comparison between flowers pollen and corbicular pollen shows that the most representative species are *Eucalyptus globulus*, *Brassica napus*, *Caesalpinia spinosa* y *Zea mays*". "Also, the more frequent pollen colors was fine yellow or orange dust for floral pollen and brown, yellow or orange for corbicular pollen, however it was figured out a variety of colors in the species *Verbena officinalis*, *Prunus serotina*, *Cydonia oblonga* y *Retama sphaerocarpa*".



## Pollination and Bee Flora

PP-187

### Plant-Pollinator Derived Biodiversity Monitoring: Leveraging Community-Based Sourcing of Bee Data for a Scalable, Granular, and Novel Approach

James T. Wilkes, Max Runzel

HiveTracks, Inc. Creston, North Carolina, United States of America

The strength of biodiversity in terrestrial ecosystems relies upon intricate dependencies between key species. Within complex food webs, pollination and the relationship between flowering plants and pollinators play a pivotal role. Although plant-pollinator interactions are widely recognized for enhancing numerous ecosystem services and functions, HiveTracks adopts a targeted model to demonstrate the correlation of these interactions with the state of biodiversity and to evaluate the impact of these interactions by quantifying the relative change in pollination events per hive and changes in the abundance of floral resources accessible to managed and native pollinators. By overcoming the challenges of scalability and providing data with a high spatial & temporal resolution, the model offers a unique approach to biodiversity monitoring by bridging the advantages of macro-level desktop assessments with the high value of ground-truthed field assessments, leveraging beekeeping communities worldwide. While each methodology and scale has its own merits and limitations, HiveTracks offers an opportunity to combine the two approaches, unlocking a brand new source of quality biodiversity monitoring.

The primary honey bee data collected by beekeepers using the HiveTracks platform includes locations of apiaries, colony counts, colony population via covered frames, harvest amounts, and images of hive configurations, brood patterns, and queens. In addition, images taken of flowering plants and native pollinators in a given region provides metadata of time and place and lays the groundwork for model-enhancing automated insect and plant recognition. We then aggregate this data and enrich it with third-party data sources such as land use, weather, and flowering data to create a data-backed assessment of biodiversity at a local scale. This assessment scales globally with each local community adopting the HiveTracks technology platform. In doing so, the platform offers a powerful tool for assessing changes in biodiversity over time rooted in a uniquely scalable focus: monitoring the plant-pollinator relationship. We present the biodiversity model being developed alongside the current status of the data collection tools created to date by HiveTracks while highlighting the importance of ongoing standardization efforts like BeeXML and WorldFAIR to increase the quality and adoption of community-based environmental data sourcing.

PP-188

### Host Trees for Amazonian Stingless Bees

Vera Lucia Imperatriz-Fonseca<sup>1</sup>, Luciano Costa<sup>2</sup>, Felipe Contrera<sup>2</sup>

<sup>1</sup>Sao Paulo University, Sao Paulo, São Paulo, Brazil

<sup>2</sup>Para Federal University, Belem, Pará, Brazil

Species interactions are relevant mechanisms shaping the structure of ecological communities. If central species disappear, interactions suffer disturbances that may affect the whole community. Many species are generalists in their interactions, which allows them to resist changes. Commensal interactions between stingless bees and host trees are poorly known compared to mutualistic interactions between flowers and bees. In dense forests, finding colonies is a difficult task, especially when they are high up in the trees, and this is one of the main reasons for the current state of the art about this kind of interaction. Here, we approach the developing field of commensal interactions between trees and bees. Our central question is: are there critical botanical groups for nesting? We found trees of 29 botanical families hosting 43 species of stingless bees. Among these, Fabaceae trees were far more frequent than all others, representing 41% of all findings, followed by Lecythidaceae, Burseraceae, Bignoniaceae, Sapotaceae, Malvaceae, Combretaceae, and Meliaceae, which together contained 45% of all bee nests found. The remaining 21 botanical families hosted 14% of the colonies. We found a positive correlation between the size and age of host trees and the abundance and richness of species of inquiline bees. However, the correlation was affected by the rarity of large trees, as most of them are of timber interest. *Apuleia leiocarpa*, *Vouacapoua americana*, *Alexa grandiflora*, *Bertholletia excelsa*, *Handroanthus serratifolius*, *Tetragastris panamensis*, *Terminalia amazonia*, *Carapa guianensis*, *Matisia cordata*, *Pouteria oppositifolia*, *Swartzia recurva*, *Sterculia excelsa*, *Virola surinamensis* e *Cynometra bauhinifolia* are fundamental for forest restoration and ecological corridor building programs.

PP-189

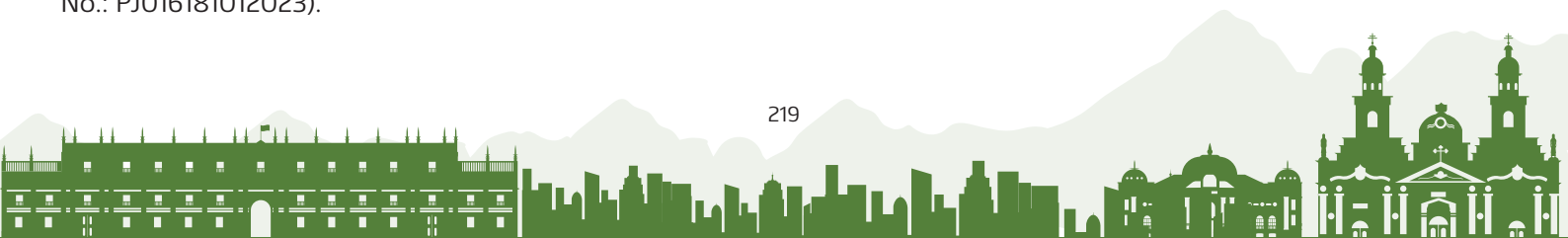
### Pollination effect by thermal insulation of honeybee hive colony box in strawberry greenhouse

Kyeong Yong Lee, Sankar Kathannan, Young Bo Lee

Department of Agricultural Biology, The National Institute of Agricultural Science, RDA, Wanju, 55365, Republic of Korea

Bee pollination is critical for strawberry production in Korea during the winter. We examined the influence of hive thermal insulation on strawberry pollination. In terms of pollination activity and hive lifespan, the average temperature inside the insulated hive was 4.6 higher than that inside an ordinary hive, and it took 20 days longer for the bees to completely disappear from the hive. In terms of bee activity, insulated hives had 2.1 times the number of bees entering the hive and 1.2 times the overall bee activity of normal beehives. On inspecting the merchantable yield and quality of strawberries to evaluate the pollination effect, the merchantable yield was 3.3% higher in the insulated hive than the general hive, and there was no significant difference in fruit quality. Therefore, we believe that hive thermal insulation is essential for strawberry pollination during the winter, as heat-insulation can improve hive internal temperature, lifespan, bee activity, and strawberry yield.

Acknowledgments: This work was supported by a grant from the National Institute of Agricultural Sciences (Project No.: PJ016181012023).



### PP-190

#### **Pyraclostrobin Fungicide Affects Gene Expression In Bees Brain Tissue *Apis mellifera* L**

**Ricardo De Oliveira Orsi, Daniel Diego Mendes, Samir Moura Kadri, Iloran Do Rosário Corrêa Moreira, Jaine Da Luz Scheffer, Aline Astolfi, Isabella De Castro Lippi, Yan Souza Lima, Marcus Vinícius Niz Alvarez**

Center of Education, Science and Technology in Rational Beekeeping (NECTAR), College of Veterinary Medicine and Animal Sciences, UNESP Sao Paulo State University, Sao Paulo, Botucatu, Brazil

Pollination by bees helps maintain balance in natural ecosystems and increase productivity in many agricultural crops, in addition to generating income for thousands of families through the production of bee products. However, there is a growing concern about the global reduction of native pollinators and honey bees in the world, one of the main reasons being the indiscriminate use of pesticides, which contaminate the environment, and consequently reduce pollinator populations. Currently, fungicides are the most detected class of pesticides in bee products, with pyraclostrobin being one of the molecules most used in agriculture and capable of causing harmful effects on bees. In the present study, the effect of ingesting the environmentally relevant dose (DAR) of the fungicide pyraclostrobin (Comet®) in *Apis mellifera* bees in the forager phase was evaluated. For this, newly emerged bees were marked and reintroduced to their respective colonies. After 21 days, these were collected and distributed into two treatments: control and pyraclostrobin. Soon after, the bees were fasted for three hours to empty the nectariferous vesicle, and the food was offered contaminated or not, with the fungicide pyraclostrobin at a RDA of 850 ppb/bee. After one and four hours of exposure, these bees were collected for brain transcriptome analysis. It was observed that bees exposed to DAR ingestion of pyraclostrobin, present alterations in the pattern of gene expression of the antioxidant system, cellular respiration, glucose metabolism and regulation of juvenile hormone and the hormone insulin. Therefore, data at the molecular level from the analysis of gene expression in brain tissue indicate that this fungicide affects the well-being of *Apis mellifera* bees in the foraging phase.

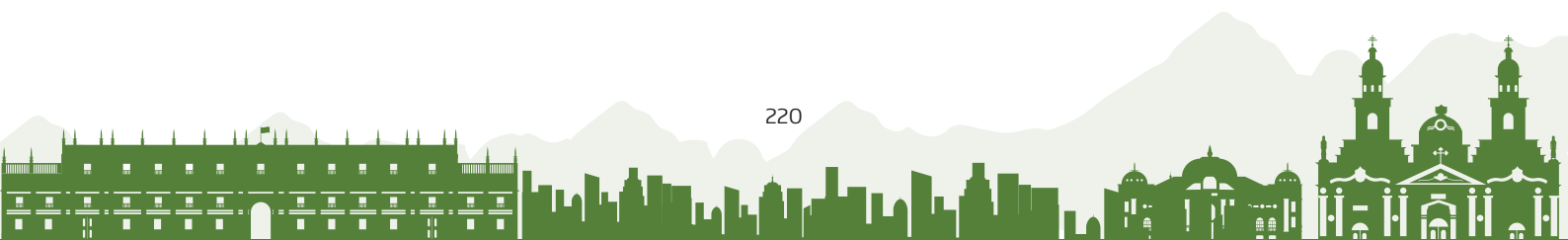
### PP-191

#### **Evidence Of Appearance For *Aethina Tumida* Murray (Coleoptera: Nitidulidae) In *Apis Mellifera* Beehives In Colombia**

**Guillermo Salamanca Grosso, Monica Osorio, Adriam Maruricio Barrera Cruzha**

Mellitopalynological Research Group, Department of Chemistry, Faculty of Sciences. University of Tolima

Throughout the world, a great diversity of species that invade *Apis mellifera* hives (Hymenoptera: Apidae) has been reported over the years. species that invade *Apis mellifera* (Hymenoptera: Apidae) hives, causing important alterations and new epizootologies and new epizootologies that affect the stability and ecosystemic dynamics of their habitat, often causing habitat, often causing important economic losses not only in biological material, but also in harvests. biological material, but also in honey and pollen harvests by reducing the productive yield. *Aethina tumida* Murray 1867 (Coleoptera: Nitidulidae), is one of those hive-invasive agents that have hives that have spread from the sub-Saharan region in Africa, throughout the world by virtue of anthropogenic activity. The objective of this work was to evaluate the evidence regarding the presence of *Aethina tumida* in *Apis mellifera* hives in Colombia. Field surveys were carried out to diagnose and identify the presence of *A. tumida* in municipalities associated with the coffee-growing zone, isolating beetle specimens when possible, and extracting them from bee colonies in the brood chamber and fruit residues near the hives. Specimens were analyzed considering taxonomic keys and morphometric parameters, taking into account 18 variables. The presence of *A. tumida* was established in the localities of Dosquebradas and Pereira, Risaralda. The keys and morphometric variables agree with the specific structure of the specimen of *A. tumida* for the head, prothorax, pterothorax, abdomen, appendages and weight, unequivocal and differentiated characters concerning *Cychramus luteus* Fabricius 1787, which evidence the presence of the etiological agent. The findings after the capture and analysis of the small beetles confirm the presence of *A. tumida* in *Apis mellifera* hives in the Colombian coffee region.





### PP-192

#### The effect of EDC (endocrine disrupting chemicals) like PFAS on pollinator health and reproductive success

Emily Bondor

Emily Bondor, Department of Horticulture, Cabrillo College Extension, Santa Cruz, California

In recent years there has been more discussion of PFAS (Per- and Polyfluorinated Substances) and their label as "forever chemicals" due to their persistence in the environment. PFAS have been found to bioaccumulate in soils and drinking waters and lab tests have shown they cause endocrine disruption in biological systems. PFAS have also been found in a number of different agrichemical products. With annual loss rates of honey bee colonies hovering around 45% in the United States, we can clearly see a big issue in overall colony health, and this invisible chemical undercurrent may be playing a larger role than we realize. Additionally, we have seen a sharp decline in honey bee queen longevity in recent decades, with elevated rates of queen supersedure. This talk will delve into potential causes of overall honey bee health declines with a focus on endocrine disruption. We will look at honey bee queen and drone fertility through the lens of a given honey bee hive's exposome.

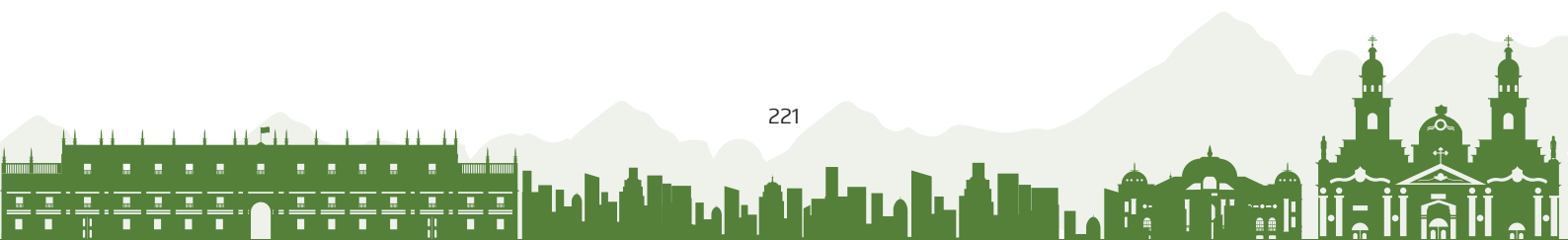
### PP-193

#### Effect of antibiotics on microbial isolates from *Apis mellifera* colonies showing bacterial disease symptoms

Samina Qamer, Beenish Javed, Muhammad Sajid

Department of Zoology, Government College University Faisalabad, Pakistan

The aim of current study is to characterize the isolates from European honeybee colonies showing EFB disease's symptoms and to determine the effectiveness of antibiotics against the EFB disease causing bacteria. For this purpose, diseased larvae samples of *Apis mellifera* showing European foulbrood symptoms were taken from two areas; Hafizabad and Sheikhpura. Effect of antibiotics from groups Cephalosporins (Velocef and Keflex) and Aminoglycosides (Kanamycin and Amikin) were studied against the isolates collected from diseased colonies. Morphological, biochemical and molecular identification of isolates was carried out. A spore forming bacterium *Bacillus paralicheniformis* was molecularly identified by using 16S rRNA gene, from isolate S4. The mean of zones of growth inhibition for all the bacterial isolates by Amikin, Kanamycin, Keflex and Velocef was 24.9mm, 24.5mm, 21.04mm, and 15.95mm, respectively. With 200µl antibiotic, MIC of Kanamycin and Amikin was 30µg, and Keflex and Velocef was 45µg. It can be concluded that Kanamycin and Amikin were found more effective to control the European foulbrood disease comparative to Keflex, and Velocef. The efficiency order of antibiotics was as follows, Amikin > Kanamycin > Keflex > Velocef.



### PP-194

#### Sub-lethal pesticide exposure in field trials may alter microbial composition of the honey bee gut microbiome

Morgan M. Cunningham<sup>1</sup>, Thomas Deckers<sup>2</sup>, Jonathan Ho<sup>3</sup>, Sydney B. Wizenberg<sup>4</sup>, Lance Lansing<sup>2</sup>, Lan Tran<sup>2</sup>, Jacqueline Zorz<sup>2</sup>, Mateus Pepinelli<sup>4</sup>, Sarah French<sup>4</sup>, Ida M. Conflitti<sup>4</sup>, Rodrigo Ortega Polo<sup>2</sup>, Shelley Hoover<sup>5</sup>, Robert Currie<sup>6</sup>, Stephen F. Pernal<sup>2</sup>, Pierre Giovenazzo<sup>7</sup>, Amro Zayed<sup>4</sup>, Leonard J. Foster<sup>8</sup>, Beecsi Consortium<sup>9</sup>, Hosna Jabbari<sup>10</sup>, M. Marta Guarna<sup>1</sup>

<sup>1</sup>Department of Computer Science, University of Victoria, Agriculture and Agri-Food Canada

<sup>2</sup>Agriculture and Agri-Food Canada, Beaverlodge Research Farm

<sup>3</sup>Department of Computer Science, University of British Columbia

<sup>4</sup>Department of Biology, York University

<sup>5</sup>Department of Biological Sciences, University of Lethbridge

<sup>6</sup>Department of Entomology, University of Manitoba

<sup>7</sup>Department of Biology, Faculty of Sciences and Engineering, Université Laval Quebec

<sup>8</sup>Department of Biochemistry and Molecular Biology, University of British Columbia

<sup>9</sup><https://beecsi.ca/beecsi-consortium/>

<sup>10</sup>Department of Computer Science, University of Victoria

In colony level field trials we determine the impact of pesticides on the gut microbiome of the European honey bee (*Apis mellifera*). The gut microbiome plays an important role in bee health and immunity; serving as an indicator of colony exposure to environmental stressors. We found a significant association between pesticide risk (proportion of LD50) in bee bodies and the microbiome composition of honey bees sampled in highbush blueberry (*Vaccinium corymbosum*) fields. In particular, we observed a positive association between the sum of pesticide risk and the relative abundance of core microbiome members: *Lactobacillus* spp. and *Bifidobacterium* spp.. These results are in alignment with cage and colony trials performed by the BeeCSI consortium that have also demonstrated agrochemical associated dysbiosis. Field trials took place across two years where colonies were situated in either highbush blueberry fields or wild forage environments and sampled throughout the pollination period. We found significant associations between pesticide risk and microbial composition in blueberry pollinating colonies. While overall correlation between risk and composition was not statistically significant in wild forage environments, similar trends for risk and individual taxa were also observed. Microbiome composition was determined by isolating the intestinal tract from sampled bees in the lab followed by shotgun metagenomics sequencing and taxonomic assignment via kraken 2 to our curated bee microbiome database. Statistical analysis included examining the correlation between pesticide and microbiome matrices via the Mantel test, as well as linear regression on the sum of pesticide risk and individual taxa abundance. This work demonstrates that sub-lethal levels of pesticide exposure may alter the composition of the gut microbiome in honey bees; targeting members responsible for core functions.

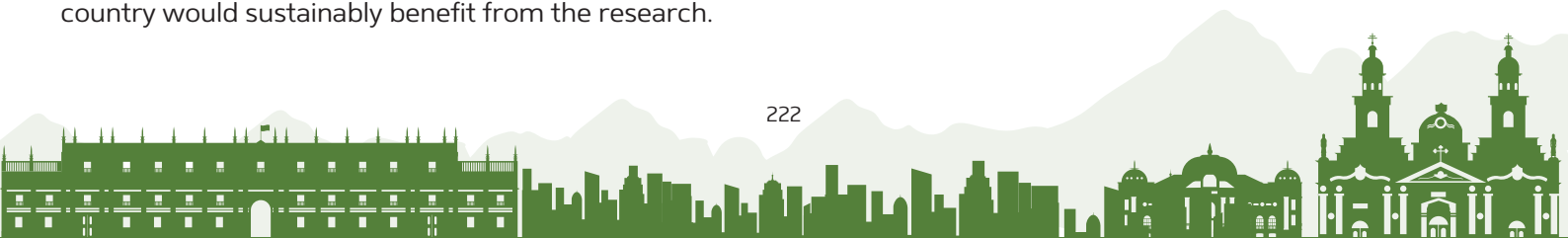
### PP-195

#### Breeding Varroa-resistant Iranian honey bee (*Apis mellifera meda*) with the intention of country-wide distribution

Pegah Valizadeh, Nader Mashayekhi Sardouiee, Gholamhossein Tahmasbi

Honey Bee Research Department, Animal Science Research Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

The parasitic mite *Varroa destructor* and the associated viruses, which are transmitted through the mite, are among the most important health challenges of honey bees globally. Most common method to deal with this challenge for several decades has been applying acaricides. However, using the chemicals for controlling *Varroa* mites has the following undesirable consequences: negative impacts on the health of honey bees and also beekeepers, increased resistance of *Varroa* mites to the acaricides, the remaining residues of the chemicals in hive products, as well as high cost of the chemicals and labor. Therefore, treatment-free beekeeping is an unavoidable alternative solution worldwide. One of the best alternatives is to create genetic resistance. In Iran, in 2016 a population of 100 colonies was selected from the National Mega Project of Breeding Iranian Honey Bees (*Apis mellifera meda*) for breeding against *Varroa*. In the last 7 years the population, which received no treatment for *Varroa*, were evaluated for honey bee survival, *Varroa* percentage, grooming and hygienic behaviors twice per year. Based on the evaluations from the best selected colonies in spring of each year new queens were bred to establish the next generation. Meanwhile, in the last 4 years applying any sort of treatments for all other pests and diseases of honey bees was also prevented in the population. Hence, the existing population is now naturally selected in tolerance against all pests and diseases of honey bees. This valuable source of untreated and survived colonies after a few more generations is expected to be used for the next level of breeding. The plan would be an organized extended breeding with the intention of a country-wide distribution of the pests and disease-tolerant Iranian honey bee queens. In this manner, the beekeeping industry of the country would sustainably benefit from the research.



### PP-196

#### Methodology to establish the presence of *Aethina tumida* Murray (Coleoptera: Nitidulidae) in honeybee hives in the Región de Los Ríos

Leticia Elena Silvestre<sup>1</sup>, Berna Barria<sup>1</sup>, Miguel Angel Neira<sup>1</sup>, Karin Gloria Barrientos<sup>2</sup>

<sup>1</sup>Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile

<sup>2</sup>Escuela de Graduados, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile.

Small hive beetle (*Aethina tumida*) is an insect which has become an important problem in honeybee hives of the European subspecies. This insect, when developing part of its life cycle inside the hives, produces economic losses to the beekeepers; due to that adults and larvae feed of pollen and honey, which causes the destruction of honeycombs and the fermentation of honey. In Chile, has been declared an exotic pest absent, however has spread reaching nearby countries such as Bolivia. This fact would suppose that is only a question of time, that could be introduced to Chile, where it is cataloged as absent which translates into non-existence of sanitary barriers that prevent their penetration, settlement, and propagation. Nevertheless, as it is a reportable pest, it is essential to develop tools that allow beekeepers to verify its possible presence in the field and thus be able to report it in a timely manner, given that there are other beetles in adult stage and moths in larval stage that can be confused with the small hive beetle. Therefore, the following study had the purpose of provider of a methodology, based on morphological characteristics, that allow detect and discriminate early the presence of *A. tumida* in honey bee hives of the Region de Los Ríos. A bibliographic review was carried out, about the morphological characteristics of the beetle. Then arthropods were captured in honeybee hives using two types of traps. Captured arthropods were classified until order level. A comparative table of morphological characters described was elaborated for each development stage and was determined the number of parameters with similar characteristics to *A. tumida*. The results of the literature review showed that the larval stage can be identified by larval type, size, color and integument processes. The adult stage, by size, color, antenna type, body shape, pronotum form, lateral view, tegument processes and exposure of abdominal tergites. Finally, in the Region de Los Rios there are a number of arthropods associated with honeybee hives that can be confused with *A. tumida* in their morphology.

### PP-197

#### Evidence of genotypic effects on the resistance of honey bees to viral infections and parasitic infestations by the mite *Varroa destructor*

Ernesto Guzman-Novoa<sup>1</sup>, Ana Karen Ramos Cuellar<sup>2</sup>, Alvaro De la Mora<sup>1</sup>, Nuria Morfin<sup>1</sup>, Francisca Contreras Escareño<sup>3</sup>, José María Tapia González<sup>4</sup>, Jose Octavio Macias-Macias<sup>4</sup>, Tatiana Petukhova<sup>5</sup>, Adriana Correa-Benítez<sup>2</sup>

<sup>1</sup>School of Environmental Sciences, University of Guelph, Guelph ON, Canada

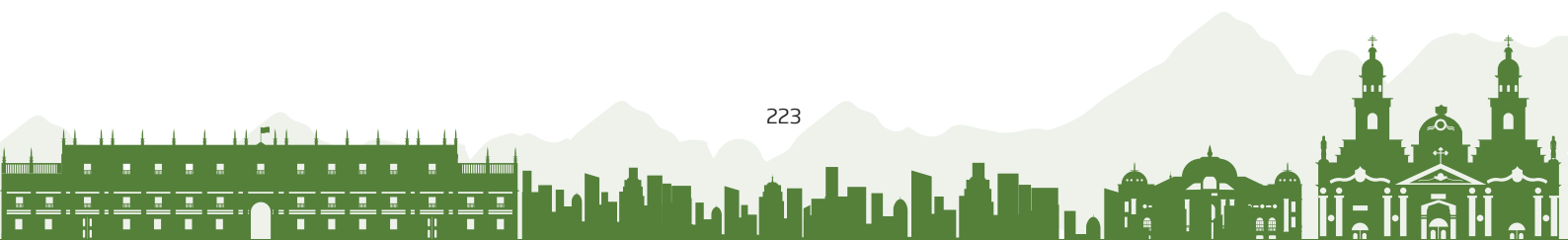
<sup>2</sup>Faculty of Veterinary Medicine, National University of Mexico, Mexico City, Mexico

<sup>3</sup>Centro Universitario de la Costa Sur, University of Guadalajara, Autlan, JAL, Mexico

<sup>4</sup>Centro Universitario del Sur, University of Guadalajara, Zapotlan, JAL, Mexico

<sup>5</sup>Dept. Population Medicine, University of Guelph, Guelph ON, Canada

The mite *Varroa destructor*, the viruses that it transmits, and the fungus *Nosema ceranae*, are among the main drivers of honey bee (*Apis mellifera*) colony losses. Therefore, it is important to find out if there are honey bee strains or genotypes that defend themselves better than others against these agents of disease. This study was conducted to analyze the effect of genotype and climate on the resistance of honey bee colonies to parasitic and viral diseases. The prevalence and levels of parasitism by *V. destructor*, or infection by *Nosema* spp., and four honey bee viruses were determined in 365 colonies of predominantly European or African ancestry (descendants of *Apis mellifera scutellata*) in subtropical and temperate regions of Mexico. *Varroa destructor* was the most prevalent parasite (95%), whilst *N. ceranae* was the least prevalent parasite (15%). Deformed wing virus (DWV) and black queen cell virus (BQCV) were the only viruses detected, at frequencies of 38% and 66%, respectively. *Varroa destructor* was significantly more prevalent in colonies of European ancestry ( $p < 0.05$ ), and the intensity of parasitism by *V. destructor* or infection by DWV and BQCV was also significantly higher in colonies of European descent than in African descent colonies ( $p < 0.01$ ), although no genotype-parasite associations were found for *N. ceranae*. Additionally, significant and positive correlations were found between *V. destructor* and DWV levels, and the abundance of these pathogens was negatively correlated with the African ancestry of colonies ( $p < 0.01$ ). However, there were no significant effects of environment on parasitism or infection intensity for the colonies of both genotypes. Therefore, it is concluded that the genotype of honey bee colonies, but not climate, influences their resistance to DWV, BQCV, and *V. destructor*.



### PP-198

#### Preliminary Study: Multilocus Sequence Typification (MLST) of Chilean Isolates of (*Paenibacillus* larvae)

Carolina Aguayo Barham<sup>1</sup>, Mauro Arancibia Zamorano<sup>1</sup>, María Esther Saldías Molina<sup>2</sup>, Jonathan Gaete<sup>1</sup>

<sup>1</sup>Laboratorio de Biotecnología, Departamento RED SAG de Laboratorios, Servicio Agrícola y Ganadero(SAG), Santiago, Chile

<sup>2</sup>Laboratorio de Bacteriología Pecuaria, Departamento RED SAG de Laboratorios, Servicio Agrícola y Ganadero(SAG), Santiago, Chile

American foulbrood is an infectious disease affecting (*Apis mellifera*) broods. Its causal agent is (*Paenibacillus* larvae) (*P. larvae*), a spore-forming bacterium. These can be transmitted through swarms, drift, or by beekeepers when exchanging equipment and hive materials. Tests that confirm the presence of (*P. larvae*), such as bacteriological culture, microscopy, biochemical tests and molecular techniques such as PCR, are used for laboratory diagnosis (OIE Manual, 2019). The use of molecular markers that allow tracing the transmission pathway of this disease is extremely necessary, given its worldwide distribution. To that end, the use of sequence-based typing (MLST) would allow for a unique, universal and unambiguous scheme that would help to better understand the spread of American foulbrood. This technique is considered a powerful tool for studying the epidemiology of pathogenic bacteria and is widely used in genotyping assays (3). In this study, 38 isolates were genotyped, coming from different regions of Chile, and 1 from abroad, and two protocols were used, one based on Krongdang et al. 2017, and another based on Morrissey et al. 2015. The results obtained, allow us to have a theory about the origin of the strain(s) acting in Chile, since it is possible to determine their difference or similarity, with respect to the strain(s) present in other countries of the world.

### PP-199

#### Effect of a liquid diet with protein, vitamins and probiotics on colony weight and immune response in *Apis mellifera iberica*

Pedro Díaz Molins<sup>1</sup>, Antonio Martínez Mateo<sup>1</sup>, Irene Muñoz<sup>3</sup>, José Serrano Marino<sup>2</sup>, María Dolores Garrido Fernández<sup>4</sup>, Daniel Dzul<sup>5</sup>

<sup>1</sup>Zukán, S.L. (Spain)

<sup>2</sup>Zoology Department. Faculty of Veterinary. University of Murcia (Spain)

<sup>3</sup>Biodiversity, Ecology and Evolution Department. Faculty of Biological Sciences. Complutense University of Madrid (Spain)

<sup>4</sup>Food Technology, Nutrition and Bromatology Department. Faculty of Veterinary. University of Murcia (Spain)

<sup>5</sup>INIFAP - Centro de Investigación Regional Sureste (Yucatán, México)

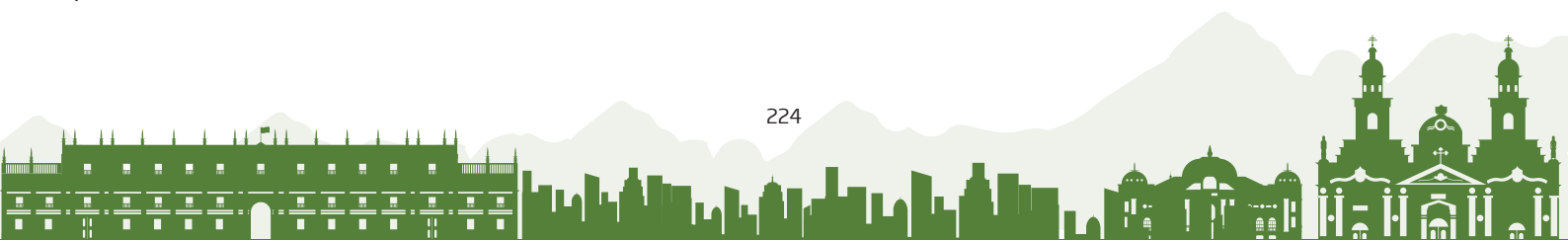
In this study we have investigated the effect of different diets, with and without added probiotics *Enterococcus faecium* y *Bacillus subtilis*, on the hive weight and the reduction of infestation caused by *Nosema* sp., in colonies of *Apis mellifera iberiensis*.

The experiments were carried out in colonies settled in Murcia (Southwest Spain) and Soria (Central Spain), between 29/10/2021 and 10/04/2022. Thirty colonies were divided into six groups, five colonies each. Three of these were fed with three food types without probiotics (groups 1, 3, 5), whereas food with probiotics was given to groups 2 and 4. Group 6 was not fed and served as control. In total, food was given 12 times each two weeks.

The different diets were almost equally consumed in all groups. Average consumption was high and stable in Murcia, whereas it was initially low in Soria (cold winters) but raised to almost 100% at the onset of spring. We found an increase in the weight of the colonies fed in both locations, compared to the hives of the control group. Food type 2 (with probiotics) exerted the best effect but this was not so with food type 4 (which also included probiotics).

The improvement of immune system was measured by counting the number of *Nosema* spores as indicator. In Murcia, after feeding, the number of spores notably increased in colony groups fed with probiotics (2 and 4), but the numbers rapidly decreased to reach the same level of null or low infestation levels found for the other groups. It seems that the rich food of groups 2 and 4 initially favored an explosive population increase of *Nosema* but that the parasite infestation was lately controlled by the improved immune system. In Soria we found similar results.

In conclusion, our initial data suggest that the inclusion of probiotics in artificial diets has favorable effects for the colonies and the investigated parameters of colony weight increase and the reduction of infestation levels of *Nosema* sp.



### PP-200

#### Preventing Colony Collapse Disorder with the Use of Enteronormin (Buzok)

Nadiya Boyko<sup>1</sup>, Oleksandr Mizernytskyi<sup>2</sup>

<sup>1</sup>RDE Centre of Molecular Microbiology and Mucosal Immunology, Uzhhorod National University, Ukraine;

<sup>2</sup>"SGP MBS" Ltd, Kyiv, Ukraine

In recent years, the global population of bees has significantly decreased, and this has become a global issue. Scientists have identified several factors that contribute to the Colony Collapse Disorder (CCD) of bees, including pesticide poisoning, acarapidosis, American rot, European rot, varroa, tropilalelaps, and aethina tumida. In addition, disorders of the healthy gut microbiome also play a role in the decline of bees. However, no single cause for CCD has been found yet.

Ukrainian scientists have developed a method to prevent CCD. The use of effective antiparasitic drug, which also have antibacterial effects, can cause intestinal dysbiosis in bees, which negatively affects their physiology. Therefore, the scientists have developed a methodology for the integrated use of the effective organic antiparasitic plant extracts with feed Meloclin and the probiotic drug Enteronormin (Buzok) for bees.

Meloclin is an antiparasitic drug that effectively treats bees against various parasites without causing any detrimental effects on their gut microbiome. Enteronormin (Buzok), on the other hand, is a probiotic drug that helps in maintaining the healthy gut microbiome of bees. The combination of these two drugs helps in the prevention of CCD by ensuring that bees are protected against parasites while maintaining their healthy gut microbiome.

The use of Meloclin and Enteronormin (Buzok) in bees has several benefits. Firstly, the combination of these two drugs helps in maintaining the healthy gut microbiome of bees. Secondly, it provides an environmentally safe way of treating bees against parasites, without causing any detrimental effects on the environment. Thirdly, it helps in the prevention of CCD by ensuring that bees are protected against parasites.

In conclusion, the decline in the population of bees has become a global issue, and CCD is one of the factors that contribute to this decline. However, Ukrainian scientists have developed a method to prevent CCD by using the combination of Meloclin and Enteronormin (Buzok). This ensures that bees are protected against parasites while maintaining their healthy gut microbiome, thus preventing the occurrence of CCD.

### PP-201

#### Effect of varroacide essential oils on the peripheral olfaction of *Apis mellifera*

Carolina Sosa Labadie<sup>1</sup>, Guillermo Bragunde<sup>1</sup>, Carmen Rossini<sup>2</sup>, Andrés González<sup>2</sup>

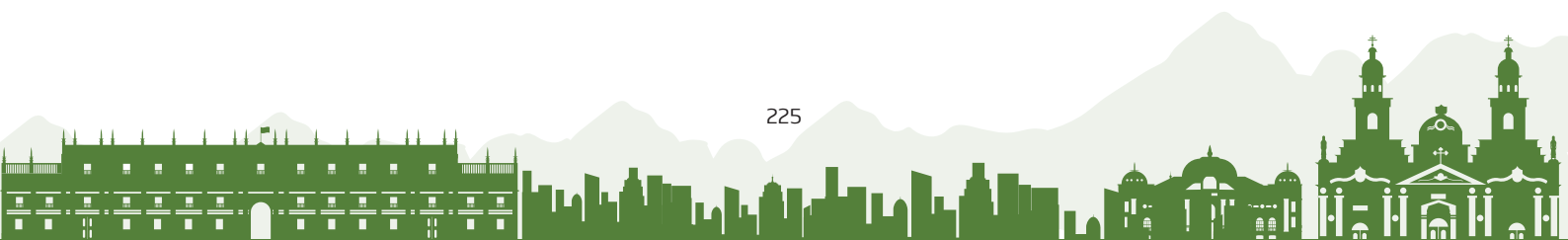
<sup>1</sup>Laboratorio de Ecología Química, Facultad de Química, UdelaR, Montevideo, Uruguay; Programa de Posgrados de la Facultad de Química, UdelaR, Montevideo, Uruguay.

<sup>2</sup>Laboratorio de Ecología Química, Facultad de Química, UdelaR, Montevideo, Uruguay.

The different diets were almost equally consumed in all groups. Average consumption was high and stable in Murcia, whereas it was initially low in Soria (cold winters) but raised to almost 100% at the onset of spring. We found an increase in the weight of the colonies fed in both locations, compared to the hives of the control group. Food type 2 (with probiotics) exerted the best effect but this was not so with food type 4 (which also included probiotics).

The improvement of immune system was measured by counting the number of *Nosema* spores as indicator. In Murcia, after feeding, the number of spores notably increased in colony groups fed with probiotics (2 and 4), but the numbers rapidly decreased to reach the same level of null or low infestation levels found for the other groups. It seems that the rich food of groups 2 and 4 initially favored an explosive population increase of *Nosema* but that the parasite infestation was lately controlled by the improved immune system. In Soria we found similar results.

In conclusion, our initial data suggest that the inclusion of probiotics in artificial diets has favorable effects for the colonies and the investigated parameters of colony weight increase and the reduction of infestation levels of *Nosema* sp.





### PP-202

#### Enteronormin-Iodine-Se complex (Buzok): design, mechanisms of action, application efficacy data

Nadiya Boyko, Oleksandr Mizernytskyi

RDE Centre of Molecular Microbiology and Mucosal Immunology, Uzhhorod National University, Ukraine; "SGP MBS", Kyiv, Ukraine

Enteronormin originally had been constructed to prevent colitis and mastitis in different farm animals and poultry, domestic small animals like cats and dogs. Today we can confirm its efficacy to maintain the bees' health.

There are at least two modern triggers influencing on the health of bees families': climate changes and massive environmental pollution. Using naturally acting mechanisms of nutrition and immune balance regulation we are protecting bees' making them less susceptible or even resistant to different contamination and infections' agents.

We designed and manufacturing multicomponent Enteronormin-Iodine-Se complex – the system of synergic commensal bacterial strains from Bacillus, Enterococcus and Lactobacillus genera, Selenium and Iodine ions as mineral elements and prebiotic components including plants' bioactive extracts and chitosan as natural origin sorbent in order to promote bees' health families. Novel generation synbiotic Enteronormin-Iodine-Se (Buzok™) is widely and successfully implemented in Ukraine and approved by FDA in USA.

The main practical approaches of Enteronormin-Iodine-Se preparation is smart packing designed for easy-to-use "ex tempore" application of live bacterial components mixed with mineral elements and other soluble ingredients. Since combined architectural content of synbiotic Enteronormin-Iodine-Se (Buzok™), the all components had been studied separately and together in order to prove their synergy and complex mechanisms of action. The bacilli and lactobacilli are responsible for the antagonistic activity against numerous agents of opportunistic infections – E.coli, Serratia, Klebsiella, Candida, Enterobacter, etc. Enterococcus is fixing proteolytic properties of Bacillus-Lactobacillus, being resistant to low pH, by production of bacteriocines, and metabolites: lactate, acetate it modulating gut microbiota composition. The all strains are able specifically modulate and normalize local immune response detected on animal models by stimulation of anti-inflammatory cytokines and production of secretory IgA in combination with effect of prebiotics compounds and added microelements.

Such direct and indirect modulation of healthy bees' microbiome can be applicable tool apart with engineering genetic modification by using of commensal Snodgrassella alvi with RNAi induced genes to deliver virus infection to Varroa mites, and can be potentially used for protection and treatment against Tropilaelaps clareae.

To keep bees healthy, to prevent timely different bees diseases resulting in great honey productivity benefit.

### PP-203

#### Oxidative Stress Biomarker to Assess the Early Impact of Environmental Pollution on Honeybees - A Novel Tool

Précillia Cochard<sup>1</sup>, Benjamin POIROT<sup>1</sup>, Andrea Cavagnino<sup>2</sup>, Martin Baraibar<sup>2</sup>

<sup>1</sup>APINOV, Research and Training Center, 10 rue Henri Bessemer, 17140 Lagord, France

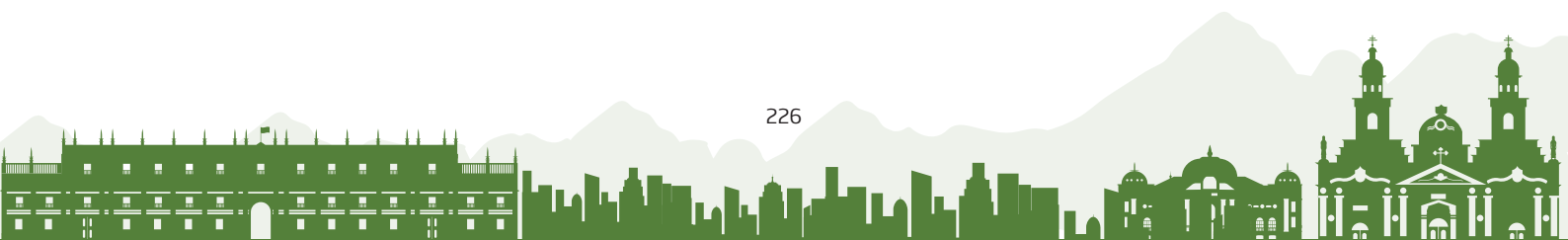
<sup>2</sup>OxiProteomics, 2 rue Antoine Etex, 4ème étage gauche, 94000 Créteil, France

Bees play a critical role in pollination and maintaining biodiversity, and their health is vital for agricultural production and ecosystem health.

Our study investigates the impact of a mixture of pesticides and polycyclic aromatic hydrocarbons (PAHs) on the health of bees. Bees have long been recognized as bioindicators of environmental quality, as they have the ability to bio-accumulate various pollutants and high sensitivity to various pollutants. However, most studies have focused on the impact of individual pollutants rather than a mixture of pollutants.

Previous research has primarily focused on the toxic effects of individual pesticides on bees, with limited investigation into the sub-lethal and long-term impacts of a combination of pollutants. This study aimed to evaluate the impact of chronic ingestion of a mixture of pesticides and PAHs by measuring the oxidative stress in bees through protein carbonylation (oxidation). The obtained results shown that protein carbonylation (oxidation) is a subtoxic biomarker, which identifies early impacts of pesticide. Moreover, this study also investigates the impact of PAHs on honeybees, which has not been extensively studied, providing a more comprehensive understanding of the stressors that bees face in their environment.

The findings of this study offer key information for the development of strategies and measures aimed at reducing pollution and preserving the health of bees, which play a critical role in pollination and maintaining ecosystem health. Moreover, this study underscores the importance of a global biomarker, such as protein carbonylation, that can evidence the effect of a cocktail of pollutants, rather than focusing on individual molecules in isolation. This approach can be used to assess the efficacy of measures aimed at reducing pollution in urban and rural areas improving environmental quality offering new environmental monitoring solutions.



### PP-204

## Impact of queen laying during oxalic acid treatment against varroa mites on efficacy and the honeybee fat body under tropical conditions

Robin Azemar<sup>1</sup>, Benjamin Poirot<sup>1</sup>, Maëlys Benard<sup>2</sup>, Précillia Cochard<sup>1</sup>, Magdi Fridmann<sup>3</sup>

<sup>1</sup>Apinov, Research and Development, 10 Rue Henri Bessemer, 17170 Lagord, France

<sup>2</sup>Université de la Réunion, 15 avenue René Cassin, 97744 Saint Denis, France

<sup>3</sup>Miel de Bourbon, 24 rue Auguste Técher 97432 Ravine des Cabris, Ile de la Réunion, France

Since 2017 Varroas destructor has been present on the Reunion island. Quickly after its arrival a part of the livestock of the island was decimated. In that context, oxalic acid was chosen as a medicine by beekeepers to manage this pest. However, besides the beneficial impact on the varroas, oxalic acid has, as every drug, deleterious impact on bees. The goal of this study is to determine the oxalic acid efficacy depending on queen laying control protocol in tropical climates. The impact of the treatment is also studied on bees health.

A varroa treatment with oxalic acid strips, Oxistrips® was used during a 42 days period with a renewal at 21 days. 1 control (the queen was laying normally) and 2 queen laying protocols were used on 5 beehives each. For the queen laying protocols, a broodless period was made by caging the queens during the 21 first days of treatment; a broodless period was made by replacing the queens by a queen cells at day 0 of the treatment.

For each protocole, the varroa population in every colony was followed by a varroas phoretic counting every 10 days during all the protocole period. To determine the impact of the treatment on the health of the bees, three measurements of the fat body of the working bees was done during the test period.

The results show that making the colony broodless, whether by encagement or by replacement of the queen by a royal cell is significantly more efficient than when the queen is laying normally. In the same way, the results concerning the bee fat bodies highlight a benefit with the protocole with broodless colonies over the protocole with a queen laying. To consolidate these results, a study with a larger workforce should be implemented. This study opens up opportunities for varroa treatment with oxalic acid in a tropical environment.

### PP-205

## Abscisic acid, a promising feed technology that boosts overwinter colony survival

Paulo Damian Mielgo, Max Watkins

Vita (Europe) Ltd

Bees play a critical role in pollination and maintaining biodiversity, and their health is vital for agricultural production and ecosystem health.

Colonies of honey bees all over the world, but particularly in North America, have been collapsing and dying during the winter period.

In Canada, the national winter loss, including non-viable bee colonies, was 45.5% with provincial losses ranging from 15.3% to 57.2%. The national colony loss reported in 2022 is almost twice the average of annual losses reported between 2007-2021 (25.8%).

In the USA, over the entire year (April 2021 – April 2022), beekeepers in the United States lost an estimated 39.0% [31.5 – 47.9] of their managed honey bee colonies. Meanwhile in (2020 – 2021) the estimated annual loss was 50.8% [30.8 – 63.1]), which was the highest annual loss on record over the last 11 years.

It has been previously demonstrated that a supplement of abscisic acid (ABA), a natural component of nectar, pollen, and honey, increases honeybee colony survival overwinter.

The aim of this work was to investigate the real effect of abscisic acid in a specific formulation for honey bees (VitaFeed Natural Boost, produced by Vita Bee Health)

Studies were performed in the USA and Canada over 3 years and the results show a strong positive effect of abscisic acid helping colonies to survive the winter period.

The average results obtained were:

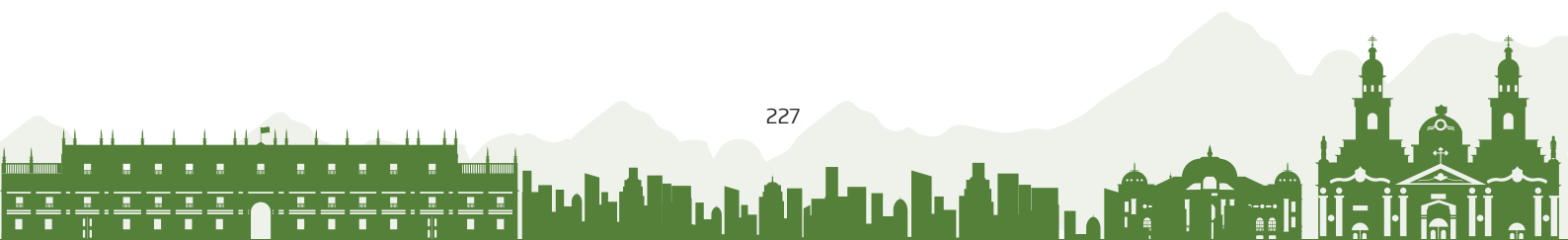
Overwintering survival: VITAFEED NATURAL BOOST: 81% / CONTROL: 49%

Overwintering population reduction: VITAFEED NATURAL BOOST: 35% / CONTROL: 59%

Adults bees increase in spring: VITAFEED NATURAL BOOST: 108% / CONTROL: 75%

Brood increase in spring: VITAFEED NATURAL BOOST: 267% / CONTROL: 198%

Abscisic acid (applied as the honey bee specific formulation 'VitaFeed Natural Boost'), has a positive influence on honeybee immunity. There is a clear reduction on the colonies lost during wintertime, one of the most negative parameters in the Apiculture industry and there is a clear positive result (cost – benefit) in the colony development (bees and brood), which will boost the pollination industry, offering a better pollination service and higher income.



### PP-206

#### Automated Detection of Human Speech within In-Hive acoustic monitoring systems: Towards Improved Beehive Health Analysis

Mahsa Abdollahi<sup>1</sup>, Ségolène Maucourt<sup>2</sup>, Pierre Giovenazzo<sup>2</sup>, Tiago H. Falk<sup>1</sup>, Evan Henry<sup>3</sup>, Nico Coallier<sup>3</sup>

<sup>1</sup>INRS-EMT, University of Quebec, Montréal, Canada

<sup>2</sup>Biology Department, Laval University, Québec, Canada

<sup>3</sup>Nectar Technologies Inc, Montréal, Canada

For many years, beekeepers have primarily relied on visual inspections to examine their beehives. Recently, the development of automated beehive monitoring tools, driven by advancements in Internet of Things has given rise to a new field known as "precision beekeeping." Over the past decade, numerous studies have focused on collecting data, such as temperature, humidity, and audio, using sensors placed inside the beehive. Of the various modalities employed for monitoring honeybee colonies, acoustics has proven to be highly versatile, enabling the detection of swarming, queen absence, and hive strength prediction. Notwithstanding, the audio modality is very sensitive to external factors, which could degrade signal quality and interfere with automated beehive monitoring. Representative examples can include the noise made by wind, rainfall, nearby vehicles, (e.g., tractors or trains), or the voices of beekeepers in the background. The latter has been shown to be particularly degrading for automated beehive monitoring systems.

Detection of speech is an active field in telecommunications for many years and so-called voice activity detectors (VAD) have been widely deployed. The use of these systems for speech collected within a beehive, however, has never been explored. In this study, we test the efficacy of three VAD methods, i) a classical energy-based method, ii) the VAD available with the WEBRTC platform for voice over internet protocol (VoIP), and iii) a recent method based on a convolutional recurrent deep neural network (CRDNN). We conduct experiments on two datasets, one is the open-access Nu-hive dataset and the other is a dataset collected at a roof-top hive at Nectar Technologies Inc (Montreal, Canada) for a period of two years. Experimental results show that WEBRTC outperforms the other methods in detecting human speech noise within bee buzzing sounds; a speech detection accuracy of 68.89% and 74.44% is seen on the Nu-hive and Nectar datasets, respectively. By fine-tuning the pretrained CRDNN model on a subset of the Nu-hive data, it could achieve an accuracy of 61.55% and 63.34% on Nu-hive and Nectar datasets, respectively. While the obtained results are promising, further work is still needed before automated audio-based beehive monitoring systems can be widely deployed.

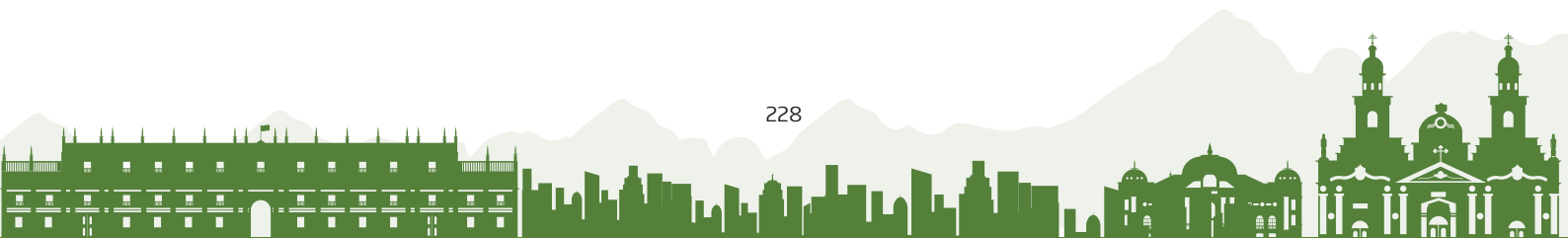
### PP-207

#### Propolis feeding modifies the expression of immune genes in challenged honey bees by injection with *Paenibacillus* larvae

Enzo Dominguez, Silvina Quintana, Sandra Karina Medici, Maria De La Paz Moline, Paloma Moran Giardini, Liesel Gende, Natalia Damiani

1.Centro de Investigación en Abejas Sociales. Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM-CONICET-UNMDP). Centro de Asociación Simple CIC-PBA. Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata Centro Científico Tecnológico CONICET - Mar del Plata. Mar del Plata, Argentina.

Bees do not seem to consume propolis under natural conditions, despite having a similar composition of polyphenols to that of pollen and nectar. These compounds have been shown to be active against bee pathogens such as the bacteria *Paenibacillus* larvae and the mite *Varroa destructor*, and to stimulate the expression of immune genes in worker bees when they are added to the nest. Overexpression of genes related to detoxification and reduction of fat bodies have also been observed in bees under forced ingestion. The gene expression of Toll18Wheeler, abaecin, hymenoptecin, vitellogenin (Vg), and domeless was determined using RT-qPCR, with the beta-actin gene as control, in bees fed for 10 days on a sugar and protein diet with (+P) and without (-P) propolis soft extract, and then subjected them to different immune treatments: Control (C), cutaneous injection (S), and injection of *P. larvae* (PL). The results showed that honey bees fed with propolis consumed significantly less food than control bees during the 10-day feeding ( $p=0.037$ ). Besides, both feeding and treatments affected the relative expression of all studied genes, and there was an interaction between the main factors. Bees in the +P/PL and +P/S groups showed lower relative expression of abaecin and hymenoptecin than bees in the -P/PL (abaecin:  $p=3.36e-06$ ; hymenoptecin:  $p=0.002$ ) and -P/S (abaecin:  $p=1.78e-13$ ; hymenoptecin:  $p=3.45e-13$ ) groups, respectively. Moreover, feeding with propolis modified the expression of the Vg gene in response to bacterial injection. These results suggest that supplementation with propolis influences the regulation of immune system pathways in immune-challenged bees. This study contributes to answering the question of whether bees would benefit from consuming propolis under natural conditions



### PP-208

#### Nectar and pollen from flower strips on crops protected by pesticide is safe for bees

Zbigniew Koltowski<sup>1</sup>, Grzegorz Doruchowski<sup>2</sup>, Krzysztof Rudziński<sup>3</sup>

<sup>1</sup>The National Institute of Horticultural Research, Apiculture Division, Pulawy, Poland

<sup>2</sup>The National Institute of Horticultural Research, Agroengineering Department, Laboratory of Plant Protection and Fertilization Technology, Skierniewice, Poland

<sup>3</sup>The National Institute of Horticultural Research, Food Safety Laboratory, Skierniewice, Poland

One of the elements of increasing biodiversity in agricultural fields is the introduction of flower strips. However, the question arises whether the chemical protection of the main crop will not be harmful to insects using the forage available in the flower patches.

The experiment to determine the exposure of pollinating insects used the flower strips on a wheat plantation was set up in the experimental fields of the National Institute of Horticulture Research in Skierniewice in 2020. The treatment with using insecticide on wheat (Decis Mega 50 EW – 0.125 l/ha) was performed during the flowering of phacelia (July 6), in the evening (after 19:00), in cloudy weather conditions, at a temperature of 18°C, average relative humidity of 72.5% and average wind speed of 1.0 m/s. The next morning, samples of nectar by pipette method were collected from *Phacelia tanacetifolia* flowers of plants growing in the first (to 2 m) and second strips (2-4 m) from the outskirts of the wheat plantation. Pollen loads from insects (mainly bumblebees) randomly caught on phacelia were also collected. The samples were chemically analyzed for deltamethrin content by gas chromatography with a double mass detector.

The results of deltamethrin residues in the phacelia flowers at the maximum level of 0.014 mg/kg in nectar and 0.040 mg/kg in pollen did not exceed the permissible residue level for honey and bee products of 0.05 mg/kg (code no. 1040000 according to Commission Regulation (EU) 2018/832 of 5 June 2018). These amounts were below the lethal concentration of deltamethrin for bees LC<sub>5</sub> (concentration for 5% bee mortality), which should be taken as the oral limit harmful concentration for these insects and is 21.6 (16.5-26.3) mg/L (Dai et al. 2010).

The obtained results indicate that the drifting with the wind of insecticides harmful to bees from the main crop to the flower strips foraging by bees, does not risky to bees and does not pose a risk of exceeding the permissible level of insecticide residues in honey and other bee products.

### PP-209

#### Screening new compounds against small hive beetles (*Aethina tumida*) with a novel acute toxicity bioassay and field trial

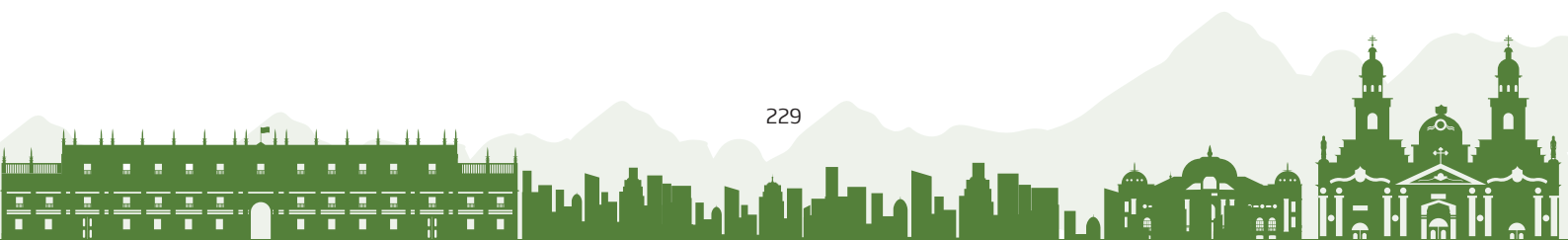
Kaylin Kleckner<sup>1</sup>, Alessandra De Carolis<sup>2</sup>, Cameron Jack<sup>1</sup>, Charles Stuhl<sup>3</sup>, Giovanni Formato<sup>2</sup>, James D. Ellis<sup>1</sup>

<sup>1</sup>Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

<sup>2</sup>Apicoltura, Produzioni e Patologia Delle Api, Istituto Zooprofilattico Sperimentale Delle Regioni Lazio e Toscana, Rome, Italy

<sup>3</sup>Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida, USA

Beekeepers need new registered products to control the small hive beetle (SHB), *Aethina tumida*, a significant pest of western honey bee (*Apis mellifera*) colonies. Few approved chemical control options exist and those available are often not effective. Here, we developed a novel acute laboratory bioassay and field trial that delivers compounds of interest to adult SHBs via pollen. We assessed the efficacy of the active ingredients coumaphos (only approved in-hive treatment for SHBs in the U.S.), acetamiprid (frequently used to control other beetles), and fipronil (commonly used in urban pest baits and off-label by beekeepers to control SHBs). Adopting our bioassay, we found acetamiprid (LC<sub>50</sub> = 2.05 µg/g) to be more toxic to SHBs than was coumaphos (LC<sub>50</sub> = 125 µg/g), yet less toxic to SHBs than was fipronil (LC<sub>50</sub> = 0.18 µg/g). In our field trial, colonies treated with acetamiprid and fipronil had significantly reduced ( $p < 0.001$ ) SHB populations over those of control colonies. Traps containing acetamiprid retained significantly higher ( $p < 0.001$ ) numbers of dead SHBs than did traps containing fipronil. From our results, we consider acetamiprid to be a promising control agent against SHBs. Future research is needed to assess the effects of acetamiprid on honey bee health, hive products, and surrounding environments.



### PP-210

#### Greenhouse environments weaken bee activity, impair physiology and unbalance intestinal microecology

Zhenguo Liu, Baohua Xu, Lufeng Liu, Ying Wang, Hongfang Wang, Xuepeng Chi

College of Animal Science and Technology, Shandong Agricultural University, Taian, Shandong 271018, People's Republic of China

Beekeeping, the key link to maintain the sustainable development of agriculture, are subjected to many negative impacts of climate change, monoculture, large temperature difference. Indeed, facility agriculture provides diversified consumption of human food, profiting from the bee pollination. However, the poor health status of bees has been restricting the economic benefits of facility agriculture. How to evaluate and improve the health level of pollination bees in facility agriculture has gained increasing attention recently. A Smart Beehive System using Internet of Things (IoT) technology were applied to continuously monitor the activities of bee colonies *Apis mellifera* for more than 100 days in strawberry greenhouses. Furthermore, the changes in gut microflora, virus load, antioxidant capacity were also detected. The results showed that the colony populations were decreased significantly ( $P < 0.05$ ). By comparing the composition of intestinal microflora, the abundance of *Gilliamella*, *Snodgrassella*, and *Pantoea* increased significantly ( $P < 0.001$ ), and *Bartonella*, was significantly decreased ( $P < 0.05$ ), despite no significant difference in the abundance of *Lactobacillus* and *Morganella* ( $P > 0.05$ ). Meanwhile, we found a significant increase in the load of bee deformed wing virus (DWV) and chronic bee paralysis virus (CBPV) ( $P < 0.05$  for DWV and  $P < 0.01$  for CBPV). Besides, the antioxidant genes of honeybees were detected and the relative expression levels of CYP9Q1 ( $P < 0.001$ ), CYP6AS14 ( $P < 0.001$ ), CYP306A1 ( $P < 0.01$ ), and CRBXase ( $P < 0.001$ ) were significantly decreased, indicating that exposure to the greenhouse environment would lead to an increase in the risk of honeybee disease and a decrease in the ability of honeybees to resist oxidation, thereby affecting the health of honeybees.

### PP-211

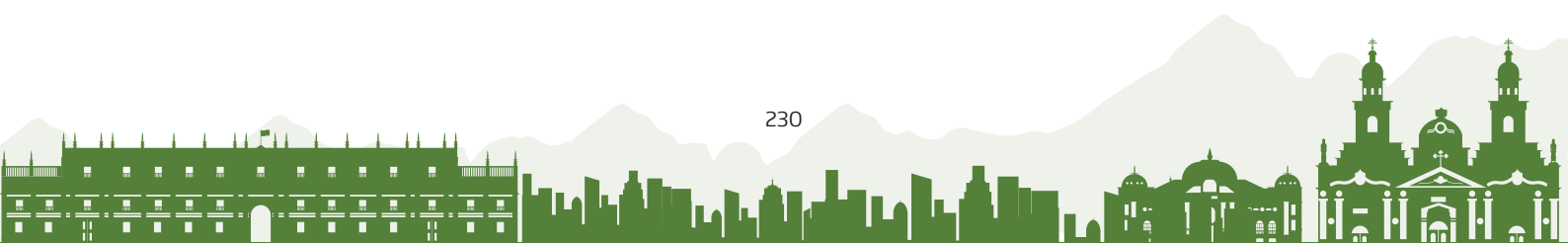
#### Status of honeybee health in Santa Catarina State, southern Brazil

Andre Amarildo Sezerino<sup>1</sup>, Tânia Patrícia Schafaschek<sup>2</sup>

<sup>1</sup>EPAGRI - Caçador Research Station, Caçador, Santa Catarina, Brazil

<sup>2</sup>EPAGRI - Videira Research Station, Videira, Santa Catarina, Brazil

An ongoing study (2021-2024) has evaluated the health of bees in apiaries in Santa Catarina state, southern Brazil. The goal of this study is to diagnose the main diseases and to establish the best approach to reduce the mortality of bees in apiaries. The study is being carried out in 10 municipalities covering the six mesoregions of the Santa Catarina State (Grande Florianópolis, North, West, South, Itajaí Valley and Highlands), each with unique climatic characteristics. Agricultural extensionists, researchers and beekeepers from each regions were trained to determine the incidence of *Varroa destructor* mite, the microsporid *Vairimorpha* sp., and clinical symptoms of other diseases such as American foulbrood, European foulbrood, the presence of small hive beetle among others. Bee samples were collected and observations were made in three bee colonies in each of the 10 locations, at three times of the year (April, July and November), in 2021 and 2022. Incidence of *Varroa* infestation ranged from 0.0% to 22.6%, with an average of 4.4% in the State. As for nosemosis, the number of infected bees (prevalence) ranged from 0.0% to 100.0% of the evaluated bees. The number of spores per bee varied from 0.0 to 17,400,000.0, with an average in the State of 779,817.2. No clinical symptoms of other diseases or the presence of the small hive beetle were observed. The results of the first two years indicate higher *Varroa* infestations in the colder regions (Campos Novos, Caçador and São Joaquim) and *Vairimorpha* sp. in warmer and more humid regions (São Pedro de Alcântara and São Miguel do Oeste). There is also a difference in infestation averages between months and years in different locations. With the results of this research, it is expected to generate a database of the situation of beekeeping health in the mesoregions of Santa Catarina State over the years, allowing the collection of data for the direction of public policies and the use by beekeepers, companies and scientific community.





### PP-212

## Evaluating the seasonal efficacy of commonly used chemical treatments on *Varroa destructor* population growth in honey bee colonies

Cameron Jack<sup>1</sup>, Humberto Boncristiani<sup>2</sup>, Cody Prouty<sup>3</sup>, James Ellis<sup>1</sup>

<sup>1</sup>Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

<sup>2</sup>Inside The Hive Media & Consulting Inc., Odenton, MD, USA

<sup>3</sup>Department of Entomology, University of Minnesota, St. Paul, MN, USA

Most beekeepers control *Varroa destructor* by treating honey bee colonies with synthetic miticides. The aim of our research was to determine how commonly used chemical treatments influence *V. destructor* population growth rates seasonally so that specific recommendations on product efficacy can be made. We applied eight chemical treatments following labeled rates to each of four seasonal (spring, summer, fall, winter) cohorts of honey bee colonies to determine how much the mite population growth rates were influenced by the treatments. The chemical treatments/formulations tested were: Apivar® (amitraz), Apistan® (tau-fluvalinate), Apiguard® (thymol), MAQS® (formic acid), Checkmite+® (coumaphos), oxalic acid (dribble), oxalic acid (shop towels), and amitraz (shop towels soaked in Bovitraz®). The Apiguard®, MAQS®, oxalic acid (dribble), and Bovitraz® treatments were effective at reducing *V. destructor* populations for at least two months during winter and spring. Unfortunately, only the Bovitraz® treatment was effective at controlling *V. destructor* in the summer and fall. Of the two amitraz-based treatments, the off-label Bovitraz® treatment was highly effective, being the only treatment to reduce *V. destructor* populations successfully in all seasons. Conversely, the commonly used treatment Apivar® was only effective in winter. The seasonal treatment efficacy data gathered through this study will allow for the refinement of treatment recommendations for *V. destructor*, especially regarding seasonal efficacy of each miticide and the temporal efficacy post-treatment.

### PP-213

## Sublethal effects of the insecticide imidacloprid on stingless bees *Scaptotrigona bipunctata* exposed for 48 hours

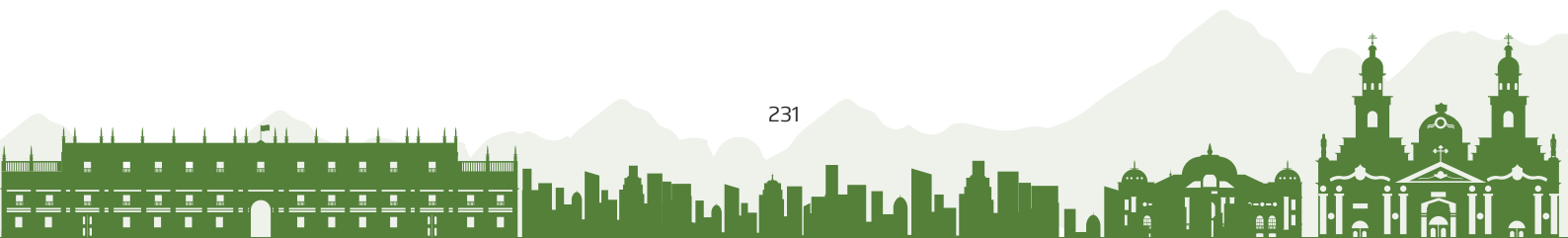
Nathalia Rodrigues da Silva de Carvalho<sup>1</sup>, Vagner de Alencar Arnout de Toledo<sup>2</sup>, Wingly Santos Beltrame<sup>1</sup>, Lucas Costa Cabral<sup>3</sup>, Adriana Aparecida Sinópolis Gigliolli<sup>1</sup>, Maria Claudia Colla Ruvolo-Takasusuki<sup>1</sup>

<sup>1</sup>Department of Biotchnology, Genetics and Cell Biology, Universidade Estadual de Maringá, Maringá, Brazil

<sup>2</sup>Department of Zootechnics, Universidade Estadual de Maringá, Maringá, Brazil

<sup>3</sup>Department of Biochemistry, Universidade Estadual de Maringá, Maringá, Brazil

The stingless bees *Scaptotrigona bipunctata* are gathered in the Meliponini tribe and are among the most bred bees in three Brazilian biomes (Pampa, Atlantic Forest, and Pantanal). Like other bee species, meliponines are declining due to factors, such as habitat fragmentation, pollution, and the indiscriminate use of agricultural pesticides. Neonicotinoids are neurotoxic insecticides widely used all over the world, and their bioavailability can cause a series of sublethal effects to exposed bees. Considering the importance of bees for pollination, meliponiculture, and the toxicity of neonicotinoids, the present work aimed to determine the LC50 (Lethal Concentration 50), the morphological changes in the midgut, and the nuclear basophilic response of brain cells chromatin of bees that were orally exposed for 48 hours to different concentrations (0.336 mg a.i./L, 1.68 mg a.i./L, 3.36 mg a.i./L, and 5.04 mg a.i./L) of the neonicotinoid imidacloprid. To this end, histological analysis of the midgut, and the histochemical technique Critical Electrolyte Concentration (CEC) of the brain were made. Initially, the LC50 was estimated for 48 hours of oral exposure to imidacloprid (1.82 mg a.i./L), in order to determine the sublethal concentrations investigated. The concentration/response correlation was positive. In the chromatin structure, an increase in CEC value was detected, which points to chromatin condensation in the brain cells of the analyzed worker bees. The midgut of treated bees showed detachment and vacuolization of epithelial cells indicative of cell death, as well as detachment of the basal lamina epithelium, disappearance of intestinal folds and relaxation of longitudinal muscles. Thus, oral exposure of stingless bees *S. bipunctata* to sublethal concentrations of the neonicotinoid imidacloprid for 48 hours may cause changes on the histological level in the midgut, and chromatin condensation with probable gene inactivation. These observed changes may result, in the long term, the death of the contaminated workers and posterior death of the colony, hence contributing to the decline of pollinators.



### PP-214

#### Dynamics on gut microbiome of honeybees based on bee diets

Jeong Hyeon Lee<sup>1</sup>, Hyun Jee Kim<sup>2</sup>, Youn Sung Choi<sup>1</sup>, Hyung Wook Kwon<sup>1</sup>

<sup>1</sup>Department of Life Sciences, Incheon National University, 119 Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea

<sup>2</sup>Convergence Research Center for Insect Vectors, 119 Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea

The gut microbiome affects health condition to host animals. Recent studies have shown that microbiome in the honeybee gut play important roles in metabolism, immunity, growth and development. The dysbiosis of the gut microbial community by unbalanced diet and biotic and abiotic stress damage tremendously honeybee health. This study focused on the gut microbiome dynamics of honeybees based on various diets using NGS method. A honeybee's diet has an effect on the diversity of the microbiota in gut, according to an analysis of alpha diversity. A total of 5 phyla, 80 genera and 120 species have been identified. Five core bacterial species of honeybee were found, but *Lactobacillus kunkeei* group and *Bombella intestini* group were identified only in bees fed with diets compared to non-fed bees. *Bombella* is known to play a role in inhibiting the growth of fungal pathogens and protecting the honeybee from infection. This research showed that alternative bee diet can be used to improve bee health by affecting microbiome dynamics.

### PP-215

#### Effect of protein supplementation on bee health

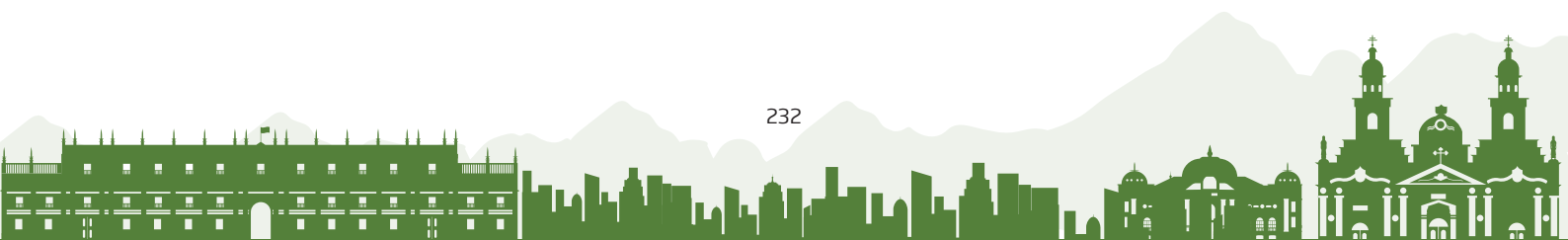
Fina Gonell Galindo<sup>1</sup>, Yaiza Gimeno<sup>2</sup>, María Fernanda López Climent<sup>3</sup>, Antonio Gomez Pajuelo<sup>1</sup>, Enrique Fuertes<sup>2</sup>, Marta Pitarch Bielsa<sup>1</sup>

<sup>1</sup>Pajuelo Consultores Apícolas S.L., C/Sant Miguel, 14. 12004, Castellon, Spain

<sup>2</sup>Asociación Defensa Sanitaria Apícola de Teruel. Apartado de Correos 52, Monreal del Campo, Teruel, Spain

<sup>3</sup>Department of Biology, Biochemistry and Natural Sciences, Universitat Jaume I, 12071 Castellon, Spain

In last decades, various factors have negatively been affecting the beekeeping sector worldwide, such as change in land use and climate change, reducing plant biodiversity and, therefore, the natural sources of nectar and pollen for the bees. Consequently, bee colonies can have nutritional imbalances, increasing the negative impact of different diseases, which leads the loss of productivity and, finally, of hives. Nutritional supplementation in times of scarcity can prevent malnutrition and its consequences. In this study, the effect on the health of bees of four complementary feeds with different protein levels have been evaluated. Two of them had a content of protein less than 10 %, and the other two more than 10 %. The essay was carried out during the months of July and August 2022, a period of the year with high temperatures and low rainfall and a moment of possible nutrient scarcity (low pollen diversity), in hives of local bees (*Apis mellifera iberiensis*) located in two areas of central Spain (Guadalajara) with presence of lavender and sunflower crops. The effect of these feeds was evaluated in three ways: measuring the strength of the colonies (population and reserves), the levels of fat body and proteins in nurse bees at the beginning and at the end, and gene expression of vitellogenin gene (vg) and a gene related to the immune system (defensin). Results demonstrate, in general, a positive impact of complementary feeds rich in protein by improving fat body levels in contrast with the control group (without protein supplementation); these feeds also induced gene expression of vg and defensin. These results show that the viability and health of the colonies in moments of nutritional stress could be improved with protein supplementation. However, further studies are needed to optimise its use.



### PP-216

### Use beebread in protein supplements to increase attractiveness for strengthening *Apis mellifera* hives

Joyce Mayra Volpini Almeida Dias<sup>1</sup>, Michelle Manfrini Morais<sup>2</sup>, Rogério Aparecido Pereira<sup>3</sup>, Andresa Berretta<sup>3</sup>, David De Jong<sup>4</sup>

<sup>1</sup>AgroBee Solutions in Pollination and Sustainability, Ribeirão Preto, Brazil

<sup>2</sup>Departament of Ecology and Evolutionary Biology, Federal University of São Paulo, Diadema, Brazil

<sup>3</sup>Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

<sup>4</sup>AgroBee Solutions in Pollination and Sustainability, Ribeirão Preto, Brazil; Apis Flora Industrial and Commercial Ltd, Ribeirão Preto, Brazil

Bees are attracted to the materials that they collect and consume by their odors. Many different apparently nutritious protein diets for bees have been developed and tested by researchers and beekeepers; however, even when placed in the hive above brood combs, bees do not always consume them. As beebread is the natural food for nurse bees in the hive, we tested the odor of this substance as an attractant. A Y-test apparatus was used with three options: (A) entrance, (B) an artificial diet formulation containing sugar cane yeast, soybean meal, rice bran, and sucrose, sufficient sucrose syrup to give it a pasty consistency, and (C) the same dry diet ingredients mixed with freshly collected beebread diluted in sucrose syrup, giving a formulation that included approximately 0.1% beebread. Five grams of each diet was placed in plastic containers covered with fine mesh wire. Five hundred newly emerged bees were marked on the thorax and returned to the hive. After four days, 10 marked bees were collected per day and individually placed at entry A of the "Y" tunnel during five days. The preference of each bee was observed for 30 minutes. The diet with beebread was significantly more visited than the diet without beebread and the entrance of the Y maze. This significance was maintained throughout the experiment, though it tended to increase after the third day. By mixing a very small amount of beebread into the protein diet, the attractiveness of the diet was significantly increased. Subsequently, after finding that the odor of beebread helps attract bees in the hive to protein diet formulations, we analyzed the volatiles of beebread to identify those that are most attractive to the bees. We have also fermented artificial protein diets with beebread organisms and found that diet consumption and protein production by nurse bees is increased.

### PP-217

### Contact contamination of honey bees in controlled fungicide spraying using fine and coarse droplets

Cássia Regina de Avelar Gomes<sup>1</sup>, Tainá Angelica De Lima Freitas<sup>1</sup>, Aline Yukari Kato<sup>1</sup>, Mariana Avelar Gomes Silva<sup>6</sup>, Yara Martins Molina Ferraz<sup>1</sup>, Thaís Regina Ramos Alves<sup>1</sup>, Jéssica Aparecida Serafim<sup>5</sup>, David De Jong<sup>4</sup>, Evandro Pereira Prado<sup>3</sup>, Eduardo Festozo Vicente<sup>5</sup>, Daniel Nicodemo<sup>2</sup>

<sup>1</sup>Post Graduate Program in Animal Science, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil.

<sup>2</sup>Department of Animal Science, São Paulo State University (Unesp), College of Agricultural and Technological Sciences, Dracena, Brazil.

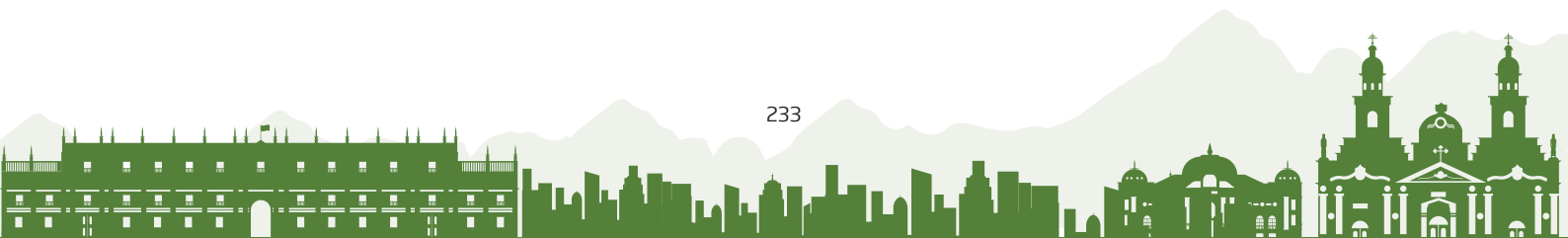
<sup>3</sup>Department of Plant Production, São Paulo State University (Unesp), College of Agricultural and Technological Sciences, Dracena, Brazil.

<sup>4</sup>Department of Genetics, University of São Paulo, Faculty of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil

<sup>5</sup>Department of Biosystems Engineering, São Paulo State University (Unesp), School of Sciences and Engineering, Tupã, Brazil

<sup>6</sup>College of Agricultural of Andradina (FCAA), Educational Foundation of Andradina (FEA), Andradina, Brazil.

In evaluations of damage caused to honey bees by pesticides, the used doses are generally based on studies of acute oral and contact toxicity tests or on determination of observed doses in honey, pollen and bees obtained from beehives. However, it is not possible to affirm that bee contamination occurred as a result of the correct use of pesticides, with respect to the doses, mode of application, and environmental conditions. The objective of this work was to quantify three fungicidal active ingredients (a. i.) in honey bees affixed on flowering soybean and cotton plants, with applications carried out in a controlled environment and in accordance with the agronomic recommendations for the use of the commercial fungicide Fox® Xpro. The applications were performed using a stationary spraying simulator equipped with a four-nozzle bar that produced fine and coarse droplets. Bees were affixed at the top and middle of the plants. A modified Quechers method was used for sample preparation and the quantification of each a. i. was performed using a HPLC. In soybean spraying, bees were contaminated with 1.09 and 7.03 µg per individual of bixafen and trifloxystrobin, respectively. The presence of prothioconazole was not detected in bees affixed to soybean plants. We observed 1.21, 0.90 and 6.89 µg per bee of bixafen, prothioconazole, and trifloxystrobin, respectively, on cotton plants. Contamination was higher when fine droplets were used compared to coarse droplets for all three a. i. Regarding the position of bees on plants, contamination was higher in bees affixed on the top compared to those affixed at mid-height for bixafen and trifloxystrobin. The determination of pesticide contamination in bees considering pesticide application criteria can be useful for assessing the risks that can affect these beneficial insects.



### PP-218

#### Intra-colony transmission of waxborne honey bee viruses following irradiation and storage

Stephen F. Pernal<sup>1</sup>, Megan J. Colwell<sup>2</sup>, Robert W. Currie<sup>2</sup>

<sup>1</sup>Agriculture & Agri-Food Canada, Beaverlodge Research Farm, Beaverlodge, Alberta, Canada

<sup>2</sup>Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada

There is building evidence that wax plays a role in virus transmission within honey bee colonies. Our investigations have shown that the abundance and prevalence of waxborne Black Queen Cell Virus (BQCV) and Deformed Wing Virus (DWW) from colonies having died the previous winter could be reduced after exposure of the comb to electron beam irradiation or by storing it for periods without bee contact. To evaluate changes in viral quantity and their potential transmission to pupae and adults, we introduced newly drawn wax comb, contaminated with BQCV, DWW-A, DWW-B and Israeli Acute Paralysis Virus (IAPV), into 60 nucleus colonies. Prior to introduction, frames were either subjected to electron beam irradiation, left untreated, or were subject to a period of storage. Our study showed that detections of viruses present on newly drawn wax decreased with a 40 kGy irradiation treatment at levels similar to when it was stored for more than 35 days. The quantities of viruses in both worker pupae and adults were also directly related to the amounts of viruses remaining on comb from colonies with high to medium virus loads, and substantially greater than the same life stages reared on comb with low levels of detectable viruses. Although storage and irradiation were similar in their ability to decrease waxborne viruses compared to comb receiving no treatment, neither technique completely eliminated transmission of viruses to pupae or adults. Our research highlights the emerging role that wax comb plays in the epidemiology of honey bee viruses.

### PP-219

#### First molecular detection of viruses in Panamanian honey bees ("*Apis mellifera*")

Yuliana Christopher<sup>1</sup>, Ruth Del Cid<sup>4</sup>, Eyleen Vega<sup>1</sup>, Hermógenes Fernández Marín<sup>1</sup>, Yuliana Christopher<sup>2</sup>, Yuliana Christopher<sup>3</sup>, Yuliana Christopher<sup>4</sup>, Hermógenes Fernández Marín<sup>5</sup>

<sup>1</sup>Centro de Biodiversidad y Descubrimiento de Drogas, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), Panamá 0843-01103, Panamá

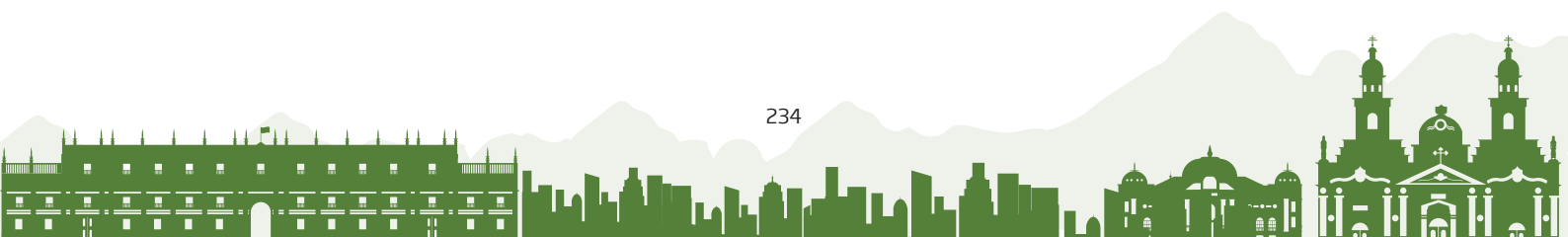
<sup>2</sup>Instituto Especializado de Análisis, IEA, Universidad de Panamá, Panamá 0824, Panamá

<sup>3</sup>Universidad Santander de Panamá, Bella Vista 7096, Panamá

<sup>4</sup>Instituto de Innovación Agropecuaria de Panamá (IDIAP), Proyecto de Investigación e Innovación Apícola de Panamá

<sup>5</sup>Sistema Nacional de Investigación, Panamá 0816-02852, Panamá

Managed and feral bees offer invaluable ecosystem services as pollinators in the agriculture systems. Some threats to honey bees ("*Apis mellifera*") include dramatic environmental pollution; climate change and a plethora of pathogens contribute to the weakening of colonies health. Recent studies have shown that Viruses, like deformed wing virus (DWW), acute bee paralysis virus (ABPV), sacbrood bee virus (SBV), and black queen cell virus (BQCV) are parasitizing honey bees in Colombia and Costa Rica, the bordering countries of Panama. This kind of study is lacking in Panama, because is an emerging honey-producer country. Here, we described the first molecular detection of viruses in Panamanian bee samples. Three different bee viruses, ABPV, BQCV, and SBV, were identified during a random screening of RNAs from asymptomatic adult bees collected in managed apiaries in four regions: Colón, Chiriquí, Herrera, and Veraguas. Also, we validated a simple, quick, and sensitive RT-PCR assay and found coinfections in colonies of the three nearest regions. Our findings will promote robust prevalence researches that evaluate the Panamanian bee's health through the country during dry and rainy seasons



### PP-220

## Isolation and characterization of bacteria from the gut of Irish honey bees for potential probiotic applications

Diana Carolina Pimentel Betancurt<sup>1</sup>, Paula Melisa Garrido<sup>2</sup>, Paul Ross<sup>3</sup>, Catherine Stanton<sup>1</sup>

<sup>1</sup>Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland

<sup>2</sup>Centro de Investigación en Abejas Sociales (CIAS), FCEyN, UNMDP, Mar del Plata, Argentina

<sup>3</sup>APC Microbiome Ireland, Biosciences Institute, University College Cork, Cork, Ireland

Honey bees are essential for pollination. Without the work they do, biodiversity and food security would be at risk. Currently the population of these insects has decreased due to the interaction of various factors such as pesticides and several diseases that affect them, among others.

The gut microbiota of honey bees is diverse and performs several important functions that significantly contribute to their nutrition, digestion, defense against pathogens, and insecticides resistance, therefore improving their immune system and health. This microbiota depends on genetic, geographic and environmental factors. The aim of this work was to isolate bacteria from the gut of Irish honey bees and to carry out their characterization, molecular identification, resistance to antibiotics and antimicrobial activity in search of potential probiotics that could mitigate the negative effects of agrochemicals and improve defense against pathogens.

For this reason, honey bees from five different colonies were collected and bacteria were isolated in MRS, MRS-cysteine and Tryptone soya agar culture medium. The characterization was performed by means of Gram staining, morphological observations and catalase test. Molecular identification was carried out by 16s rRNA sequencing. The susceptibility of the isolated bacteria to antibiotics was tested using the micro-dilution method.

Antibacterial activity against an indicator strain were evaluated by deferred antagonism assays. Forty strains were isolated from the gut of Irish bees. Analysis of the 16S rRNA gene sequence indicated that some bacteria belong to *Lactobacillus helsingborgensis*, *L. apis*, *Apilactobacillus kunkeei*, among others. Almost all bacteria were Gram-positive and catalase negative differing in colony morphology and size. Based on the antibiotic results, most of the bacteria were susceptible to all of the tested antibiotics, according to the European Food Safety Authority breakpoints.

The MIC ranged between 0.016-1.23 mg/L for erythromycin, 0.14-5.97 mg/L for tetracycline, and 0.4-3.7 mg/L for chloramphenicol. Few bacteria were found to be resistant to ampicillin. Some of strains showed antimicrobial activity against the indicator strain. The results suggest that some of the bacteria isolated from the bee gut have potential application for probiotics due to their safety (generally regarded as safe GRAS status), non-resistance to antibiotics, and their antimicrobial activity.

### PP-221 [Bee Health]

## Bacterial contamination of drone honey bee semen is influenced by the collection technique

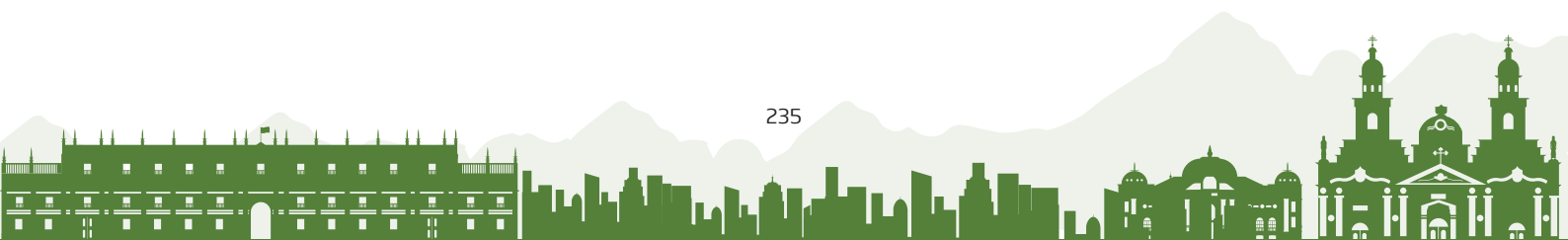
Jesús Yániz<sup>1</sup>, Pilar Santolaria<sup>1</sup>, Miguel Angel Silvestre<sup>2</sup>, Marion Toquet<sup>3</sup>, Raquel Toledo Perona<sup>3</sup>, Angel Gomez Marín<sup>3</sup>

<sup>1</sup>BIOFITER research group, Environmental Sciences Institute (IUCA), Department of Animal Production and Food Sciences, University of Zaragoza, Huesca, Spain.

<sup>2</sup>Departamento de Biología Celular, Biología Funcional y Antropología Física, Universitat de València, 46100 Burjassot, Spain.

<sup>3</sup>ProVaginBIO Research Group, Departamento Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, Carrer Tirant lo Blanc, 7, Alfara del Patriarca, Valencia, 46115, Spain

Semen can be contaminated with bacteria during collection or further processing, even when done under careful conditions. This contamination reduces sperm viability and increases the risk of infection transmission to the queen after instrumental insemination. Two main techniques for collecting semen in honey bee drones have been described, based on the dissection of the seminal vesicles and the induction of ejaculation through the application of manual pressure on the thorax and abdomen. This study was designed to study the effect of these two collection methods on bacterial contamination of semen samples. Mature flying drones of the *Apis mellifera iberiensis* subspecies were recovered from 8 hives, in the afternoon of days with good weather, on their return to the hive after blocking the entrance with a queen excluder. A total of 21 pooled samples, containing semen from 8 drones, were prepared after collection from the seminal vesicles or from the ejaculates. Analysis included bacterial culture and counts on Plate Count Agar for aerobic mesophilic bacteria, MacConkey Agar for gram-negative enteric bacteria and Blood Agar for more fastidious microorganisms. From the 42 semen samples analyzed, 28 (66.7%) were positive for aerobic mesophilic and/or gram-negative enteric bacteria, which represented 100% of the samples obtained from the seminal vesicles (21 of 21), and 33.3 % of those obtained from the ejaculates (7 of 21). No bacterial growth was observed on Blood Agar plates for the ejaculate samples, while 61.9% of the semen samples collected from the seminal vesicles showed bacterial growth. In contaminated samples, the degree of bacterial contamination was much higher for the vesicular samples, with 2.23 x 10<sup>6</sup> CFU/mL of aerobic mesophilic bacteria on average (range 0.2 to 8.2 x 10<sup>6</sup>), than for the ejaculates, with an average of 1736 CFU/mL aerobic mesophilic bacteria (range 40-3000). In conclusion, the collection method has a great impact on the degree of bacterial contamination of semen samples, being the traditional ejaculation method more favorable than the collection of semen from the seminal vesicles. This work was supported by the Spanish AEI-MICINN (grant PID2020-112673RB-I00), the DGA-FSE (grant A07\_17R) and grants RYC2021-032245-I and CIACIF/2021/245.





## Bee Health

### PP-222

## Healthy Bees Plan 2030 (HBP 2030), Working together to improve honey bee health and husbandry in England & Wales

Rebekah Jane Clarkson

National Bee Unit, Animal and Plant Health Agency

The National Bee Unit (NBU) has been involved in the management and control of bee pests and diseases, along with training and dissemination of information to beekeepers for over 60 years in England & Wales. Bee Health Programmes are delivered on behalf of Department for Environment Food and Rural Affairs (Defra) and Welsh Government (WG), and all are committed to working with stakeholders in a 10 year partnership plan to improve honey bee health and husbandry in England & Wales. The plan focusses on achieving four key outcomes:

1. Effective biosecurity and good standards of husbandry, to minimise pest and disease risks and so improve the sustainability of honey bee populations.
2. Enhanced skills and production capability/capacity of beekeepers and bee farmers.
3. Sound science and evidence underpinning the actions taken to support bee health.
4. Increased opportunities for knowledge exchange and partnership working on bee health and wider pollinator needs.

Stakeholders meet quarterly in a Forum and include the British Beekeepers Association, Welsh Beekeepers' Association, Bee Farmers' Association, National Farmers' Union, National Diploma in Beekeeping and the Veterinary Medicines Directorate. All partners agree to ensure sound science and evidence underpin actions taken to support bee health. The Forum developed an implementation plan with more than fifty actions for the partners to work on together to achieve the outcomes in the Healthy Bees Plan 2030. Key to the delivery is how stakeholders work together towards the shared goal to improve honey bee health. Joint delivery of the agreed actions allows a range of stakeholders to get involved.

Successes to date include; risk based inspections to target resources effectively, encouraging mentoring and improved communications to make beekeeping information more accessible.

### PP-223

## Application of a new *Bacillus thuringiensis* formulation (B403) against wax moth (*Galleria mellonella*) during laboratory and field trials

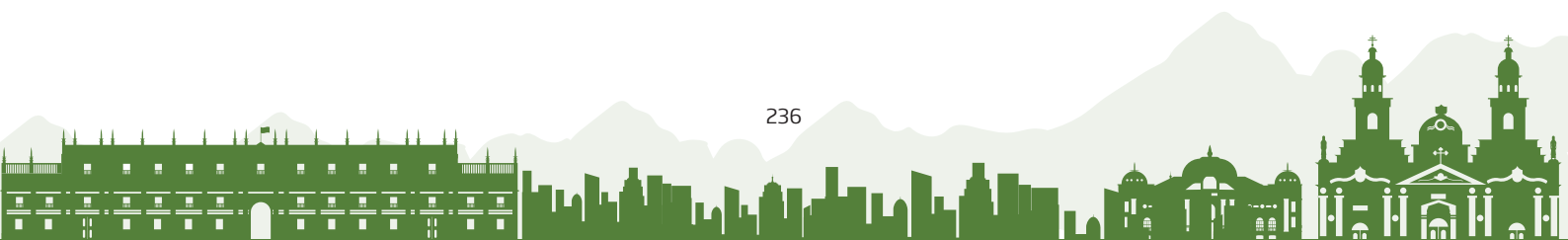
Alexandros Papachristoforou<sup>1</sup>, Konstantinos Ilanidis<sup>2</sup>, Paulo Mielgo<sup>2</sup>, Max Watkins<sup>2</sup>

<sup>1</sup>Department of Animal Science, University of Thessaly, Greece

<sup>2</sup>Vita Bee Health, Basingstoke, UK

*Galleria mellonella*, commonly known as wax moth, can destroy honeybee wax combs and induce serious economic losses for beekeepers. Some products, based on *Bacillus thuringiensis* (BT), such as B401, have been applied against wax moth larvae (WML) to protect combs. Here we describe the efficacy assessment of a new BT product, B403, against WML under laboratory and field conditions.

A culture of 96 WML was used in laboratory bioassays to test the repellency effect of B403. The study was applied as an artificial food assay, choice test. Each WML of 3rd instar was placed in a plastic container (17cm long x 12cm wide x 4cm high). A line was drawn at the center of the container to mark the treated (food application) and untreated sides. A portion of artificial diet (0.25g) was added to the one side, containing either B403 (Batch A: 10%, Batch B: 20%), B401 (Batch C: positive control) or only water (Batch D: control). Assessments were conducted for a 3-week period. Alive or dead WML were counted. The location of larvae was also scored at each assessment and their distances from food resource was evaluated. All WML were found dead in B403 20% and B401 while in B403 10%, only 4.16% survived. Almost all dead larvae were found away from the treated side. 96.84% of WML survived in control Batch D. In field trials, 16 Langstroth hive boxes, containing 10 frames each, were divided in four batches of 40. Frames were sprayed with A: 5% B401, B: 10% of B403, C: 20% of B403 and D: water (control) and then, they were placed in a storehouse easily accessible by wax moth. Four months post application, 99.65% and 99.33% of frames' area was safe for B403 20% and B403 10%, respectively. The entire area of B401 was also safe while all control frames were destroyed by WML in control Batch.



### PP-224

#### Get those fleas off my bees!: Assessing the isoxazoline class as possible treatments for *Varroa destructor* control and safety towards honey bees (*Apis mellifera*)

Julia St. Amant, Cameron J. Jack

Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

Chemical resistance to pyrethroids (tau-fluvalinate), organophosphates (coumaphos) and formamidines (amitraz) in *Varroa destructor* populations has been observed worldwide. To find new active ingredients that could reduce *V. destructor* populations in honey bee (*Apis mellifera*) colonies, we examined the acute toxicity of insecticides from the isoxazoline class of chemicals. Isoxazoline insecticides are commonly used to treat ticks and fleas on mammals through topical solutions. Here, we calculated the lethal dose that killed 50% of the population (LD50) and the selectivity ratios (honey bee LD50/*V. destructor* LD50) for afoxolaner, fluralaner, sarolaner, and lotilaner on *V. destructor* and honey bees by using direct topical application methods. These values were compared to amitraz, coumaphos, a solvent control and a positive control (dimethoate). Fluralaner (LD50 = 0.068 ng/*V. destructor*) was the most toxic isoxazoline insecticide and only 1.9x less toxic than amitraz (0.036 ng/*V. destructor*), but was still 26,200x more toxic than coumaphos (1788.692 ng/*V. destructor*). Sarolaner (selectivity ratio = 0.05) and afoxolaner (selectivity ratio = 0.12) were more toxic to honey bees than they were to *V. destructor*, while fluralaner was 126x more toxic to *V. destructor* than to honey bees. These results suggest that fluralaner could be a promising active ingredient due to its high toxicity to *V. destructor* and its relatively low toxicity to honey bees. Further research should be conducted to study the toxicity of isoxazoline insecticides, specifically fluralaner, on honey bee larvae as well as the sub-lethal impacts of these chemicals to adult honey bees.

### PP-225

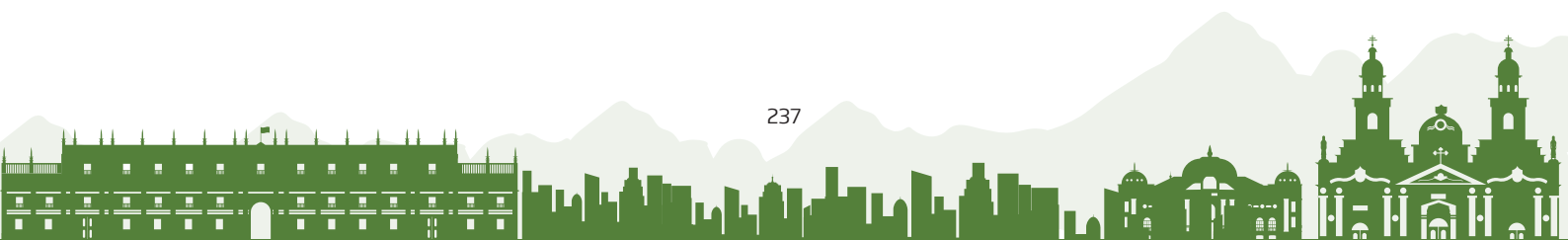
#### Supplementation with probiotics in bee feed – a complementary strategy for enhancing bee health and welfare

Adriana Cristina Urcan<sup>1</sup>, Adriana Dalila Criste<sup>1</sup>, Daniel Severus Dezmirean<sup>2</sup>

<sup>1</sup>Department of Microbiology and Immunology, Faculty of Animal Science and Biotechnology, Cluj-Napoca, Romania

<sup>2</sup>Department of Apiculture and Sericulture, Faculty of Animal Science and Biotechnology, Cluj-Napoca, Romania

Multiple causal factors negatively influence the strength of bee colonies and their probability of survival. Among them, parasites and pathogens are the main candidates, such as pesticide exposure, diet quantity, quality and diversity, unfavorable weather, and forage circumstances. All these factors may affect honeybee colonies individually or in various combinations, possibly causing severe disturbance of honeybee microbiota composition. A new strategy to improve the health of honeybees is the study of their microbiome and the use of probiotics in the treatment and prophylaxis. The aim of this study was to assess the impact of supplements with *Lactobacillus plantarum* and *Lactobacillus acidophilus* on honeybees' gut microbiota composition. We investigated the presence of these bacteria in the gut of control and *Nosema* spp. spore-infected bees. The study was conducted in laboratory-controlled conditions. The bees were fed with syrup supplemented with 2.5, 5 and 10% of each probiotic. It was found that following the probiotic administration *Nosema* spp. spore counts were significantly reduced, which may indicate that *L. plantarum* and *L. acidophilus* may be a promising food additive for *nosemosis* prevention. This result points out that continuous probiotic treatment shall change bees' gut microbiome composition and mitigate the influence of *Nosema* spp. infection. Toward these results, it is possible lactic acid bacteria supplementation may protect honeybees by improving microbiome and immune functions. Further, *in vivo*, research is needed to evaluate the bioavailability of the probiotic bacteria and its beneficial impact on the health of bee colonies.



### PP-226

#### Sterilizing royal jelly for use in Western honey bee (*Apis mellifera*) in vitro rearing programs

Jennifer M. Standley<sup>1</sup>, Adam CN Wong<sup>1</sup>, Cody Prouty<sup>1</sup>, Jose Marcelino<sup>1</sup>, James Fulton<sup>2</sup>, Allison Dehnel<sup>1</sup>, James Ellis<sup>1</sup>

<sup>1</sup>Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

<sup>2</sup>Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA

Rearing *Apis mellifera* workers in vitro is an important technique used in honey bee research. In this assay, bee larvae feed on an artificial diet that includes royal jelly (RJ), a worker bee glandular secretion often collected and sourced internationally. The use of commercially imported RJ in in vitro rearing programs could risk pathogen spread. This risk may be mitigated by irradiating the RJ prior to use, though radiation could affect RJ's utilization in an artificial diet. The purpose of this study was to determine if honey bees could be reared in vitro on a diet containing irradiated RJ and what dose of irradiation would kill bacterial and fungal inoculants. Experiment 1: We fed larvae a diet containing unirradiated RJ, irradiated RJ (25 kGy), or a temperature/storage (TS) control (RJ stored in the irradiation facility for three days, the time it takes to irradiate RJ). Larval survival to eclosion in all treatments met the U.S. Environmental Protection Agency's toxicology guidelines. Significantly fewer larvae survived to eclosion when fed a diet containing irradiated RJ (73.6%) than when fed one containing unirradiated RJ (85.7%) or the TS control RJ (81.9%). Experiment 2: RJ was inoculated with *Bacillus subtilis* (gram-positive bacterium), *Acetobacter indonesiensis* (gram-negative bacterium), or *Ascophaera apis* (causative fungal agent of chalkbrood in honey bees), then irradiated at 5, 10, 15, 20, or 25 kGy, and finally plated onto culture media. Microbial growth was quantified every 24 hours for seven (bacteria) or ten (fungus) days and compared to that of inoculated, but unirradiated RJ (control). No microbial growth occurred on plates to which inoculated/irradiated (15 - 25 kGy) RJ was added, while microbes grew on all plates in the control group. The results demonstrate that worker bees can be reared on a diet that includes irradiated RJ and that 15 kGy may be sufficient to kill bacterial and fungal inoculants in RJ. Additional diet refinements may be necessary to improve in vitro reared bee survival to levels experienced by individuals reared on a diet containing unirradiated RJ and to determine the effects of irradiation on virus contaminants of RJ.

### PP-227

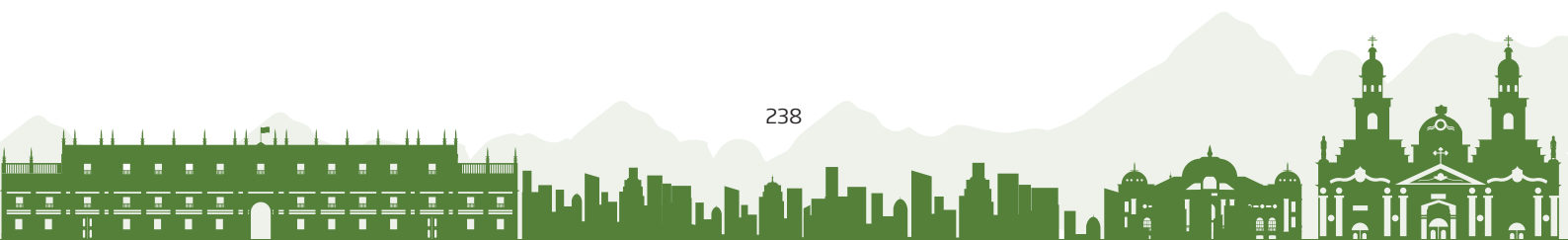
#### Assessment of the biotic communities associated with royal jelly using high-throughput metagenomics (DNA-seq) and metatranscriptomics (total RNA-seq) sequencing

Jennifer M. Standley<sup>1</sup>, Jose Marcelino<sup>1</sup>, Fahong Yu<sup>2</sup>, James Ellis<sup>1</sup>

<sup>1</sup>Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

<sup>2</sup>Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida, USA

Royal jelly (RJ) is a glandular secretion that worker honey bees feed to developing larvae and adult queens. The microbiome, virome, and other biota associated with RJ are underexplored. This is especially important given that RJ is a key ingredient in the diet fed to larvae in in vitro rearing programs and RJ used in such programs is often sourced internationally. The purpose of this study was to characterize the microbiome, virome, and other biota present in commercially sourced RJ and to compare them with microbial communities present in RJ collected from queen cells in an apiary managed at the University of Florida (Gainesville, FL, USA). To achieve this goal, twelve samples of RJ (11 purchased in the USA but internationally sourced and one collected from local queen cells) were used for the analysis. DNA and RNA were extracted using a DNeasy Blood and Tissue Kit and MagMAX Viral/Pathogen Nucleic Acid Isolation Kit. Using high-throughput metagenomics (DNA-seq) and metatranscriptomics (total RNA-seq), and parallel sequencing, we identified DNA and RNA fragments from organisms of interest in RJ. These included *Nosema ceranae*, *Paenibacillus* larvae, *Melissococcus plutonius*, *Varroa destructor*, *Aethina tumida*, *Galleria mellonella*, Acute bee paralysis virus, Slow bee paralysis virus, Sacbrood virus, and *Varroa destructor* virus, as well as multiple beneficial bacteria from the genera *Lactobacillus*, *Actinobacteria*, and *Gluconobacter*. It is important to note that these results confirm the presence of DNA and RNA fragments from these organisms in the RJ samples. They do not imply that whole replicative viruses or other viable organisms were present in the RJ. The tested RJ may have been exposed to at least fragments of the identified organisms in the hive or while processed commercially. The results present for the first time a comprehensive DNA and RNA microbial profile in RJ.



### PP-228

#### Aspects related to the activity of the hexanic extract of *Achyrocline satureioides* on *Paenibacillus* larvae, a bacterial honey bee pathogen

Diana Carolina Pimentel Betancurt<sup>1</sup>, Romina Marisa Heredia<sup>2</sup>, Maria Fernanda Paletti Rovey<sup>3</sup>, Carlos Huallpa<sup>3</sup>, Maria De Las Mercedes Oliva<sup>3</sup>

<sup>1</sup>Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland

<sup>2</sup>Department of Molecular Biology, Institute of Environmental Biotechnology and Health (INBIAS- CONICET), National University of Río Cuarto. Río Cuarto, Córdoba, Argentina.

<sup>3</sup>Department of Microbiology and Immunology, Faculty of Exact, Physical-Chemical and Natural Sciences, National University of Río Cuarto. Río Cuarto, Córdoba, Argentina.

*Paenibacillus* larvae is a spore-forming, Gram-positive bacterium that causes the devastating disease American foulbrood. This disease spreads rapidly, affecting the well-being of the hive and the apiary generating the considerable loss of this essential pollinating insect and contributing to the Colony collapse disorder (CCD). Currently, there is no cure for American foulbrood and beekeepers immediately act by burning contaminated hives.

Plant extracts are proposed as an ecological, viable and acceptable alternative for the treatment of the disease in hives. *Achyrocline satureioides* is a plant native to southeastern part of South America. It is widely used for its pharmacological and antibacterial properties. Our previous findings show that the hexanic extract (HE) of this plant inhibits the growth of *P. larvae* at relatively low concentrations.

This study investigates several hypotheses about how the HE of *A. satureioides* could act on *P. larvae*, by studying the effect of HE on bacterial growth, changes in *P. larvae* membrane by analysis of phospholipids, and fatty acids by GC-FID as well as the fluidity of *P. larvae* membrane and the changes in their morphology by electron transmission microscopy.

HE had an inhibitory effect on the growth of the microorganism. *P. larvae* presented a high percentage of saturated acids compared to unsaturated ones. HE did not generate very visible changes in the membrane of *P. larvae* during the time cells were exposed to the extract under the assessed conditions neither did it have a negative effect on membrane fluidity. Bacterial cells treated with HE, exhibited multiple damages such as cell wall degradation, alteration of the membrane and loss of cytoplasmic content. Other effects such as deformed cells, polarization of the cytoplasmic content and more lysed cells were observed compared to the control at 72 h.

The results suggested that HE from *A. satureioides* causes structural damage and possibly the different compounds present in HE affect the cell wall of *P. larvae*.

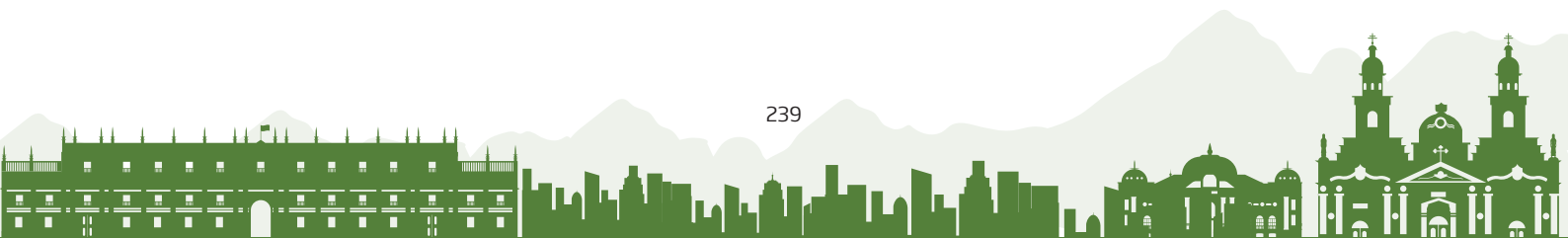
### PP-229

#### Honey Bee (*Apis mellifera* L.) Colony loss in Korea: Climate Change and Varroa destructor

Yong Soo Choi, Dae Gun Oh, Dong Won Kim, Su Bae Kim, Bo Sun Park, You Young Jo, Peter Akonhte

Department of Agricultural Biology Apiculture Division, National Institute of Agricultural Science, Rural Development Administration, Rep. of Korea

The *Apis mellifera* are extensive pollinators for agricultural production and environmental ecosystems. But unfortunately, honey bee losses over winter and autumn season of 2021-2022 and 2022-2023 in the Republic of Korea about 17% to 50% colonies. Honey bees are heavy losses in Korea cause to complex factors (climate change, varroa mite, disease and pesticide materials etc.). We were used 2 years survey of Korean beekeepers` winter colony loss and before winter loss by Varroa mite and weather condition during spring to winter season. Varroa mite was mutated to against Flivarinate pesticide chemical. The Fluvarinate chemical was used almost beekeepers in Korea during 30 years. Many beekeepers could not control Varroa mite in year. Varroa mite due to destroy immune system in honey bee body and make short lifespan. Week nursing bee cannot do wintering by varroa mite damage. Worm winter can destroy winter colony that nursing bee flying out of hive after that disappear in winter season. Presently, beekeeper should be change chemical pesticide for controlling Varroa mite and development colony management technique for overcoming climate change. Beekeepers have to do circular use chemical materials (amitras etc.) and Formic acid, oxalic acid, Drone pupa trapping etc. Some beekeepers already used smart technique like heater and sense for control temperature and humidity to against climate change for example warm winter and high daily temperature range.



### PP-230

#### Honey bee-eDNA for non-targeted monitoring of *Apis mellifera* L. microbial and arthropod communities

Leigh Boardman<sup>1</sup>, Jose A. P. Marcelino<sup>2</sup>, Rafael E. Valentin<sup>3</sup>, Humberto Boncristiani<sup>4</sup>, Jennifer M. Standley<sup>2</sup>, James D. Ellis<sup>2</sup>

<sup>1</sup>Department of Biological Sciences & Center for Biodiversity Research, The University of Memphis, Memphis, TN, USA

<sup>2</sup>Honey Bee Research and Extension Laboratory, Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

<sup>3</sup>Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA

<sup>4</sup>Inside The Hive Media & Consulting Inc., Odenton, MD, USA

Early detection of invasive species is fundamental to maintaining healthy honey bee colonies and ensuring the pollination services they provide. Traditional methods often target known pests or pathogens, and may miss new and emerging invasive species, especially if they are cryptic or do not cause visible signs of disease. Environmental DNA (eDNA), which is DNA collected from environmental samples rather than biological organisms, offers a powerful monitoring tool when paired with high-throughput sequencing. Here, we used eDNA metabarcoding to detect pests and pathogens in various sample sources collected within hives and an apiary. We collected eDNA from 13 different sample sources (e.g., hive detritus, hive tools, hive feeder, plants) to determine which sample sources had viable DNA that could be collected and analyzed. We also compared two collection methods (swabbing surfaces with moistened swabs vs. spray/wash surface aggregation). DNA was extracted and quantified before metabarcoding via high-throughput sequencing was completed using four universal primer sets: arthropod (cytochrome oxidase I and 16S), bacterial (16S) and fungal (ITS). Overall, we were able to extract sufficient DNA from most sample sources using both collection methods. As expected from visual observations of colonies within our apiary, we detected DNA from the small hive beetle (*Aethina tumida*), *Varroa destructor*, and *Melissococcus plutonius* (causative agent of European foulbrood). Several other arthropods and microbes of apiculture interest were detected. This research is ongoing as we develop eDNA metabarcoding into a comprehensive tool for colony health surveys.

### PP-231

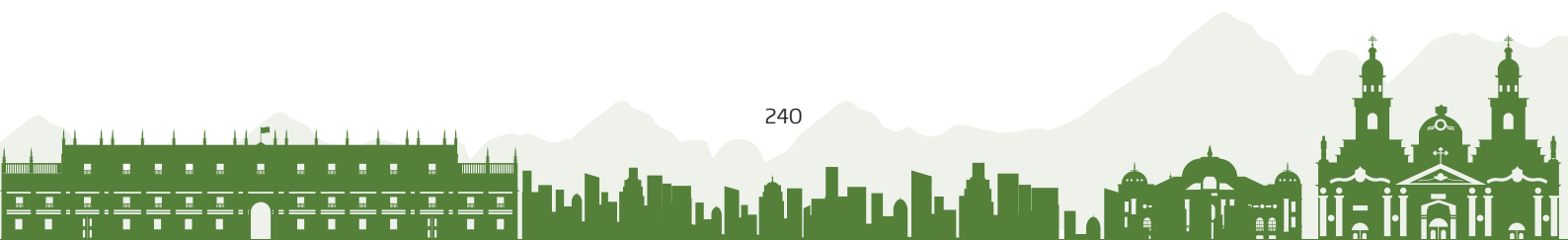
#### Evolution of Natural Mite-resistant Colonies on Hawaii

Stephen J Martin<sup>1</sup>, Scott Nikaido<sup>2</sup>, Ethel M Villalobos<sup>2</sup>

<sup>1</sup>School of Environment and Life Sciences, The University of Salford, Manchester M5 4WT, UK

<sup>2</sup>Plant and Environmental Protection Science, University of Hawaii at Manoa, Honolulu, HI, USA

The *Varroa* mite (*Varroa destructor*) in conjunction with the deadly deformed wing virus (DWV) have been responsible for large scale colony losses of managed and feral honey bees. In many parts of the world, beekeepers have been applying acaricide treatments on a regular basis for decades to reduce the mite population and decrease the viral levels in their colonies. Acaricide treatment became synonymous with colony survival. However, more and more often, researchers and beekeepers are reporting the existence of populations of *Varroa*-resistant honeybees. Natural *Varroa* Resistance, the ability to survive without treatment, has developed in honey bee populations in multiple countries including South Africa, Cuba, UK, Brazil, France, Norway and Sweden. These populations all exhibit similar behavioral traits including: 1- *Varroa* infected brood removal/cannibalism, 2- "recapping" or the repair of small holes created on the cap of brood cells that were inspected for *Varroa*, and 3-increased mite infertility. In this preliminary study we examined the recapping frequency of 36 colonies in 13 apiaries on Oahu, Hawaii. The colonies sampled were all of European descent and the majority had been obtained as local swarms or were removals from man-made dwellings, not purchased from a queen breeder. Recapping rates observed indicated that 90 % of the colonies and 100% of the apiaries exhibited Natural *Varroa* Resistance on Oahu, Hawaii. In a recent survey we determined that approximately 2/3 of Oahu beekeepers stopped treating their colonies against the mite. The reasons for this change are diverse: shipping costs to Hawaii are high and not all products are easily imported in small scale, some beekeepers had a personal dislike or distrust of chemical treatments, and some just noticed the colonies were needing intervention less frequently. The isolation of the island, paired with the selective and progressive removal of treatment in a subtropical environment where the mite is always active may have resulted in a relatively fast development of adaptive traits on Oahu.





### PP-232

## Revolutionizing Beekeeping: An innovative device for varroa mite control and hive monitoring with IOT Integration

Ron Corkidi, Avner Einav

ToBe Influencing innovations, Israel

Varroa mite infestation is considered one of the most critical threats to honeybee colonies worldwide, causing significant economic losses to the beekeeping industry. The mite feeds on bee larvae, causing physical damage, transmitting viruses, and weakening the immune system, which can ultimately lead to colony loss.

Unfortunately, conventional treatment methods are not always effective in eliminating the mite and can be harmful to the bees and the environment.

Contact strips and essential oils have limitations and disadvantages. Strips rely on the bees to carry the active compounds, and mites have developed resistance to some of the chemicals. Essential oils are not potent enough to eliminate all the mites, applying them is labor intensive and their effectiveness is temperature-dependent, which can be challenging to control.

In contrast to conventional treatments, the Hive Master is an autonomous automatic device that uses precise, micropulses of gas to eliminate varroa mites. The active compound is delivered to the mites' respiratory system using only 10% of the active compound required compared to conventional contact strips. This precision not only ensures effectiveness but also reduces the likelihood of resistance developing in the varroa population over time. By using low amounts of active compounds in the perfect timing, the Hive Master is able to eliminate the varroa without harming the bees or disrupting the hive ecosystem.

In the past year the technology has been tested in a pilot study with over a thousand hives, resulting in a 50% reduction in winter losses and a 30% increase in honey production during spring.

Furthermore, the Hive Master turns the hive into a smart hive by measuring temperature, humidity, and other factors. This information is then used to provide insights to beekeepers about the strength and health of their hive, reducing labor and time while improving the overall management of the colony. The Hive Master technology offers a promising solution to the ongoing challenge of Varroa mite infestations in honeybee colonies.

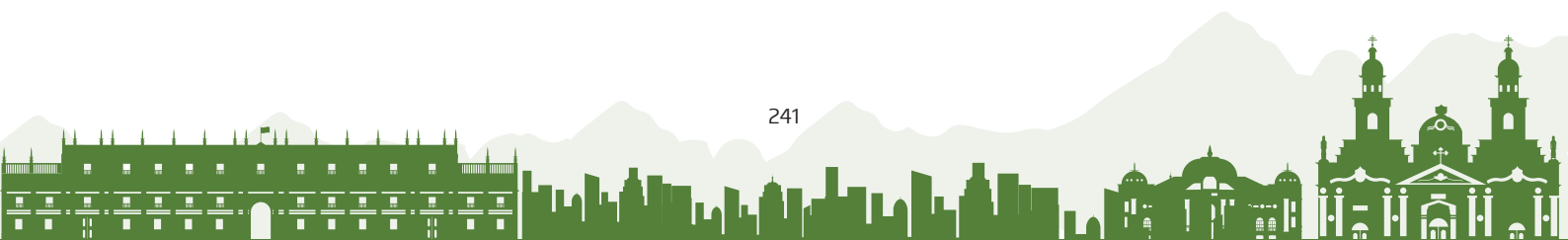
### PP-233

## Low aggression towards conspecific stingless bees exposed to a lethal biopesticide

Sircio Alison Dos Santos, André Rodrigues De Souza, Amanda Prato Da Silva, Bruno Gusmão Vieira, Fábio Santos Nascimento

Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Departamento de Biologia, Universidade de São Paulo – USP, Ribeirão Preto-SP, Brazil

Biopesticides have become popular worldwide due to their alleged environmental sustainability and effectiveness in pest control, which in turn increases the need to investigate potential harmful effects on non-target organisms like stingless bees, the main native pollinators in the Neotropics. We tested lethal and side effects of a commercial formulation (Boveril®) based on the entomopathogenic fungus *Beauveria bassiana* on the stingless bee *Scaptotrigona aff. depilis*. Topical exposure to a realistic dose of the biopesticide (5 µg A.I./ µL [ $10^4$  viable conidia/ µL]) caused mortality when applied on the thorax (TL50 = 30 min) or abdomen (TL50 = 24 h). After 12 h following topical exposure on the abdomen, the cuticular hydrocarbon profile (social recognition cues) of bees changed, as detected by gas chromatography coupled with mass spectrometry analyses. Finally, these exposed bees with altered cuticular chemical profile (nestmates or not) were less aggressed by guards at the nest entrance, as demonstrated in a lure presentation assay. Our results suggest that fungus-based biopesticides can be detrimental to pollinators, causing mortality, and increasing the probability of pathogen transmission between stingless bees colonies.



### PP-234

#### Hello nice too meet you, i wan show mi idea tested about the kill varroa, we design a special box using the solar Energy

Julio Santiago Figueroa Lillo

Julio Santiago Figueroa Lillo

Our solar greenhouse, consists a box with three spaces available, the which prevent leaving bees, closing the entrance with a ventilated mesh, in of the transparente roof closed and ventilated. This treatment Have a duration depends of the degrees celcius at the zone. And Have a emergency ventilator fan in case of exceed the temperatura for help with air and weting towel in to increase the humidity. Also Is possible do the treatment during at night for 45 min moisturizing usually by 30 min. How we have different location apiary with out electrical Energy, we supply this Energy with photovoltaic panel conected with a heater reptile with regulator temperature to arrive until the 43 degrees, these treatment Is during 45 min depends of the degrees of the day, and the zone where Is employed the apiary, normally our temperature in summer time borders 35 until 39 degrees. This factor favors shortening the Time of the treatment considerably for which the bees dont feel streessfull and the brood nest dont be afected de real jelly, and the nutrition dont see damaged for the temperature introduced for the sun rays exposed.

### PP-235

#### Contact contamination of mandaguari bees *Scaptotrigona postica* in controlled fungicide spraying

Cássia Regina de Avelar Gomes<sup>1</sup>, Tainá Angelica De Lima Freitas<sup>1</sup>, Aline Yukari Kato<sup>1</sup>, Mariana Avelar Gomes Silva<sup>6</sup>, Yara Martins Molina Ferraz<sup>1</sup>, Thaís Regina Ramos Alves<sup>1</sup>, Jéssica Aparecida Serafim<sup>5</sup>, David De Jong<sup>4</sup>, Evandro Pereira Prado<sup>3</sup>, Eduardo Festozo Vicente<sup>5</sup>, Daniel Nicodemo<sup>2</sup>

<sup>1</sup>Post Graduate Program in Animal Science, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil.

<sup>2</sup>Department of Animal Science, São Paulo State University (Unesp), College of Agricultural and Technological Sciences, Dracena, Brazil.

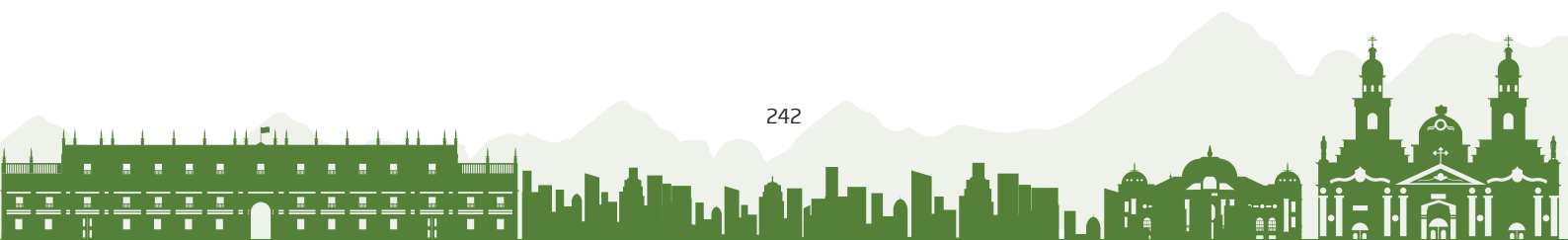
<sup>3</sup>Department of Plant Production, São Paulo State University (Unesp), College of Agricultural and Technological Sciences, Dracena, Brazil.

<sup>4</sup>Department of Genetics, University of São Paulo, Faculty of Medicine of Ribeirão Preto, Ribeirão Preto, Brazil

<sup>5</sup>Department of Biosystems Engineering, São Paulo State University (Unesp), School of Sciences and Engineering, Tupã, Brazil

<sup>6</sup>College of Agricultural of Andradina (FCAA), Educational Foundation of Andradina (FEA), Andradina, Brazil.

Fungicides are used during all stages of development of agricultural crops, even during flowering period. At this time of plant reproduction, bees are more likely to be directly contaminated and suffer immediate impacts due to acute exposure to this pesticide. The aim of this study was to determine the contamination of mandaguari bees *Scaptotrigona postica* with three active ingredients (a.i.) that make up a commercial fungicide (Fox® Xpro) under real simulation of application, carried out according to agronomic recommendations. Soybean and cotton plants were grown in pots and taken to a controlled spraying laboratory when the plants were in the reproductive stage. Mandaguari bees were affixed at the top and middle of plants and fungicide applications were made using nozzles that produced fine and coarse droplets. Samples were prepared based on a QuEChERS method with modifications and the determination of the active ingredients was performed using a HPLC. The observed dose of bixafen was 0.74 µg per bee, in both agricultural crops. Higher doses of trifloxystrobin were detected in soybean plants (2.23 µg per bee) than in cotton plants (0.64 µg per bee). The active ingredient prothioconazole was detected in only two samples of bees affixed on soybean plants (0.55 µg per bee) and one sample of bees affixed on cotton plants (0.56 µg per bee). It is suggested that the prothioconazole active ingredient was observed in lower amounts due to its low solubility and the fact that the commercial fungicide is a concentrated suspension product. In general, contamination was higher in bees affixed at the top of plants compared to those affixed at mid-height. The droplet size did not interfere with the amount of fungicide that contaminated the bees. Determining bee contamination with pesticides under simulation with ideal application conditions can contribute to better decisions regarding restrictions on pesticide use in crops.



### PP-236

#### Imidacloprid promotes oxidative stress in *Apis mellifera* larvae and pupae after oral exposure in the field

Daiani Rodrigues Moreira<sup>1</sup>, Tuan Henrique Smielewski de Souza<sup>2</sup>, Douglas Galhardo<sup>2</sup>, Cinthia Leão Figueira<sup>2</sup>, Breno Gabriel<sup>3</sup>, Francieli das Chagas<sup>1</sup>, Jean Rocha<sup>2</sup>, Alessandra Benites<sup>2</sup>, Angélica deSouza Khatlab<sup>2</sup>, Eliane Gasparino<sup>2</sup>, Vagner de Alencar Arnaut de Toledo<sup>2</sup>, Adriana A. Sinópolis Gigliolli<sup>1</sup>, Maria Claudia Colla Ruvolo-Takasusuki<sup>1</sup>

<sup>1</sup>Department of Biotechnology, Genetics and Cell Biology, Universidade Estadual de Maringá, Maringá, Brazil

<sup>2</sup>Department of Animal Science, Universidade Estadual de Maringá, Maringá, Brazil

<sup>3</sup>Department of Exact Sciences, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brazil

Bees are pollinators of different agricultural crops and can come into contact with insecticides by collecting pollen and nectar. Neonicotinoids are a class of insecticides that act on the central nervous system of insects and have the potential to cause physiological changes and consequently promote the death of pollinators. This study aimed to verify the action of the neonicotinoid imidacloprid (IMD) on the redox state of larvae and pupae of Africanized *Apis mellifera* after forty-two days of oral exposure in the field. For this, the apiary was divided into three experimental groups: control, commercial product imidacloprid (commercial IMD Evidence 700 WG®), and the active ingredient imidacloprid (IMD active ingredient pestanal®) with six repetitions per group. Every three days, 300 mL of syrup containing the insecticides at a concentration of 1 µg/L was provided. The control group received only syrup. After exposure of the colonies, the bee larvae and pupae were collected in cryogenic tubes and frozen in liquid nitrogen for enzymatic and non-enzymatic antioxidant evaluations. Larvae exposed to commercial IMD showed higher nitrite and carbonylated protein content, and lower activity of the enzymes superoxide dismutase (SOD) and catalase (CAT). The treatment with IMD active principle confirmed an increase in the activity of SOD and CAT. Regarding the content of reduced glutathione (GSH), it was observed that the larvae exposed to commercial IMD and active principle showed the lowest content of this non-enzymatic antioxidant. No significant effects of treatments were observed on total antioxidant capacity ( $p$ -value>0.05). Pupae exposed to IMD active principle showed the highest and lowest SOD and CAT enzyme activity, respectively. No treatments were significantly affected for nitrite content, carbonylated protein, GSH, and total antioxidant capacity ( $p$ -value>0.05). Thus, the long period of exposure of larvae and pupae to the neonicotinoid increased the oxidative stress state of the insects, in which, the high production of oxidative compounds can cause cell death and compromise the survival of the insect and consequently the colony.

### PP-237

#### Identification of (*Nosema Sp.*), (*Paenibacillus larvae*) and (*Melissococcus plutonius*) species by PCR in apiaries in Benin

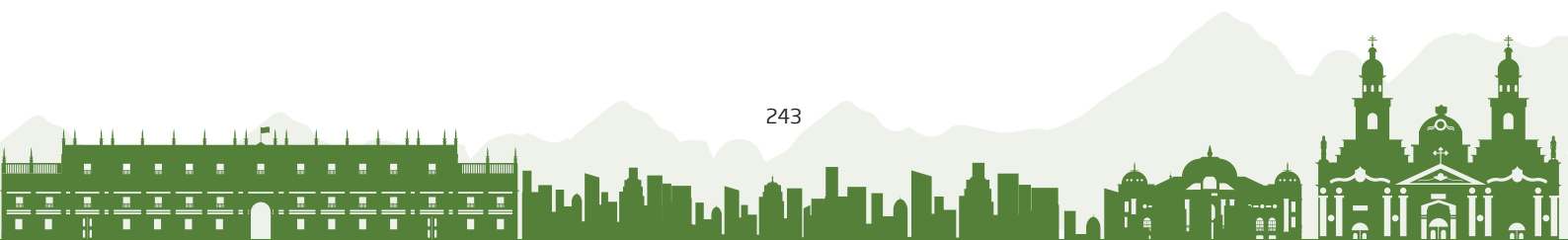
Aude Eminsédé Kelomey<sup>1</sup>, Hélène Legout<sup>2</sup>, Farid Tovignon Farid T. Badé<sup>1</sup>, Adolphe Adjanonhoun<sup>3</sup>, Lionel Garnery<sup>2</sup>, Lamine Baba Moussa<sup>1</sup>

<sup>1</sup>Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, 05 BP 1604 Cotonou, Benin.

<sup>2</sup>Laboratoire Evolution Génome Comportement Ecologie (EGCE) UMR 9191, Gif sur-Yvette

<sup>3</sup>Institut National des Recherches Agricoles du Bénin (INRAB), Direction Générale, Abomey-Calavi, 01bp 884 Cotonou, Bénin.

The honeybee (*A. mellifera* L.) is an important social insect, for its role in the pollination of crops and for the products generated in the hives. (*Nosema sp.*), (*Paenibacillus larvae*) and (*Melissococcus plutonius*) belong to the most important pests of (*A. mellifera*) Linnaeus bees (Hymenoptera: Apidae). The aim of this study is to identify the presence of (*Nosema Sp.*), (*Paenibacillus larvae*) and (*Melissococcus plutonius*) in apiaries in Benin by RT-PCR. 37 bee samples from three districts of Benin (North-East, North-West and Central) were analyzed in the study. We used PCR assays based on 16 S ribosomal RNA and primers specific to each of the parasites of interest to identify the parasites in the bee samples. Data were analyzed with graphpad prism 8 and minitab 19 software. Only (*N. ceranae*) belonging to (*Nosema Sp.*) was found in the tested samples with a rate of 13.51%. This indicates that the nosema parasite is present in the apiaries of Benin. This is the first report of *N. ceranae* in (*A. mellifera*) colonies in Benin. Intensive surveys should be carried out to determine the distribution and prevalence of (*N. ceranae*) in the different regions of Benin in order to effectively control this parasite.



### PP-238

#### Development of bioproducts for bee health: *Bacillus* based probiotics for honey bees

Vera Strogolova, Vyacheslav Strogolov

Strong Microbials Inc., Milwaukee, Wisconsin, USA

Biological products include live microorganisms. Probiotics and direct-fed microbials (DFMs) are new biological products that came into apiary in the last decade. The introduction of these biological products was informed by limited but growing body of research that established some benefits the probiotic *Bacillus* bacteria can offer for honey bee health. In 1978 USDA researcher Martha Gilliam identified 110 *Bacillus* species including *Bacillus subtilis*, and *Bacillus pumilus* in honey bee gut, in pollen, and in bee bread. Gilliam's research showed that *Bacilli* inhibit the growth of chalkbrood-causing fungal spores. *Bacilli* also secrete a significant amount of amylase enzyme in the honey bee gut to digest carbohydrates in honey and nectar. *Bacillus* is one of the dominant groups of bacteria in honey and propolis. Sequencing of propolis samples identified *Bacillus badius* and *Bacillus thermolactis* species, specifically. *Bacilli* likely play an important role in all of these niches because these bacteria produce bioactive metabolites. During 2014-2022, Strong Microbials developed and tested different formulations of commercially available probiotics for honey bees in the USA. We hypothesized that *Bacillus* bacteria can be formulated and delivered to honey bees in liquid feed (sugar syrup) or other feed (pollen or protein patties, fondant), safely and effectively, and that these biological products will promote honey bee colony health and vigor. Field data is presented to evaluate these trials, opening up new questions for the next generation of probiotics for honey bees.

### PP-239

#### Do novel biopesticides affect honey bees *Apis mellifera carnica*?

Maja Ivana Smodiš Škerl<sup>1</sup>, Tadeja Tumpej<sup>2</sup>, Katarina Karnicar<sup>3</sup>, Katarina Karnicar<sup>4</sup>, Dusan Turk<sup>3</sup>, Dusan Turk<sup>4</sup>, Janez Prešern<sup>1</sup>, Ivana Tlak Gajger<sup>5</sup>, Jerica Sabotič<sup>2</sup>

<sup>1</sup>Animal Production Department, Agricultural institute of Slovenia, Ljubljana, Slovenia

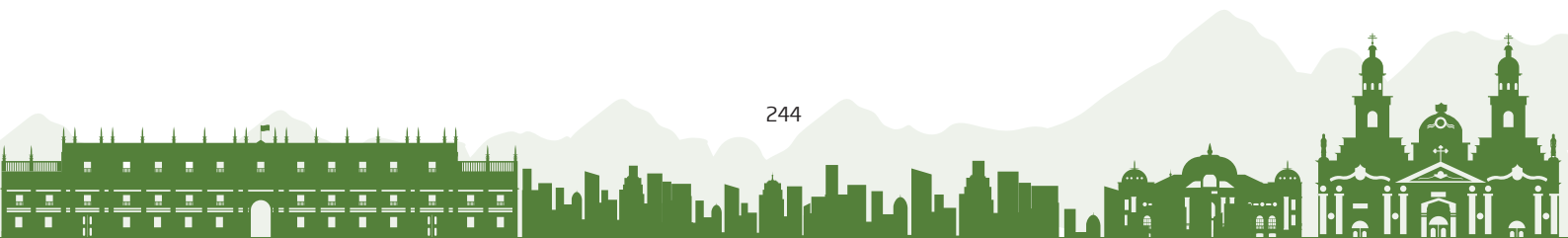
<sup>2</sup>Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia

<sup>3</sup>Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute, Ljubljana, Slovenia

<sup>4</sup>Centre of Excellence for Integrated approaches in Chemistry and Biology of Proteins (CIPKeBiP), Slovenia

<sup>5</sup>Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine University of Zagreb, Zagreb, Croatia

Biopesticides are a novel alternative to protect agricultural plants from phytopathogens. At the same time, the honey bees *Apis mellifera* are one of the most important insect pollinators of cultivated crops and wild vegetation, and therefore vulnerable to pesticide-induced impacts. The losses of honey bees caused by environmental pollutants suggest that other beneficial insects may experience a similar outcome. Biopesticides, the proteins from mushrooms, have a high potential to replace the use of pesticides and protect plants. However, are they safe for the bees? We tested several novel biopesticides on adult bees and larvae of honey bee colony. With the first test, we treated adult bees *A. m. carnica* and determined the acute toxicity of selected novel proteins in 24, 48 and 72 hrs. The purpose of our second test was to determine the chronic toxicity to honey bee larvae after repeated oral exposure before pupa life stadium. At the end of the experiment, we determined the mortality level and weight of the larvae. In both trials, we did not detect significant differences. In the next step, we will evaluate the potential damage of the midgut of treated honey bee larvae by using histological and immunohistological methods. We hypothesize that we will not find particular changes in the cell structure in the midgut and determine the low level of cell death in the larval midgut. These mentioned methods are very useful in risk assessment schemes and in scientific research with the main goal to determine and explain the acute and chronic effects of any target compound on *A. mellifera*.



### PP-240

#### Towards sustainable control methods against *Melissococcus plutonius*, the causative agent of European foulbrood

Vincent Diemann<sup>1</sup>, Florine Ory<sup>2</sup>, Alexis Beaurepaire<sup>3</sup>, Camille Ameline<sup>1</sup>, Marylaure De La Harpe<sup>1</sup>, Vincent Duchemin<sup>2</sup>, Sammy Sakhry<sup>2</sup>, Jean-Daniel Charrière<sup>1</sup>, Benjamin Dainat<sup>1</sup>

<sup>1</sup>Swiss Bee Research Centre Agroscope Liebefeld, Bern, Switzerland

<sup>2</sup>Department of Ecology and Evolution, University of Lausanne, Lausanne Switzerland

<sup>3</sup>Institute of Bee Health, University of Bern, Bern, Switzerland

European foulbrood (EFB) outbreaks have become frequent in several countries in the last decades and can lead to colony and economic losses. This was the case in Switzerland, where the number of yearly cases was multiplied by ten over a decade, affecting up to 5% of the country's apiaries. Because of the costs involved in sanitizing affected colonies and controlling neighboring apiaries for disease spread, there is a need to better understand the biology and virulence of *Melissococcus plutonius*, the causative agent of EFB and the resistance mechanisms of its host to develop sustainable method to control this pathogenic bacteria. To address these issues, we studied i) the intracolony transmission pathways of the bacteria to the larvae, ii) the bactericidal effect of the nutritive jelly fed to larvae, iii) the occurrence of transgenerational immune priming of larvae via the exposure of the mother queen to the pathogen, and iv) whether breeding honey bees for *M. plutonius* resistance is in principle possible. We found that i) the bacteria contaminating the jelly are rather of external than of internal origin, that is come from the cuticle of contaminated nurse bees entering the cells rather than of their alimentary tract through which jelly is secreted, ii) queen and worker jellies, as well as jellies from different colonies have different bactericidal properties, iii) experimental exposure of queens to viable *M. plutonius* bacteria did not protect their offspring from infection and iv) that nestmate workers differ in their susceptibility to infections depending on their father, indicating a genetically based resistance, one of the compulsory criteria needed to select a trait. Our results improve our knowledge of *M. plutonius* virulence, of the host's defense mechanisms and open avenues for sustainable control methods.

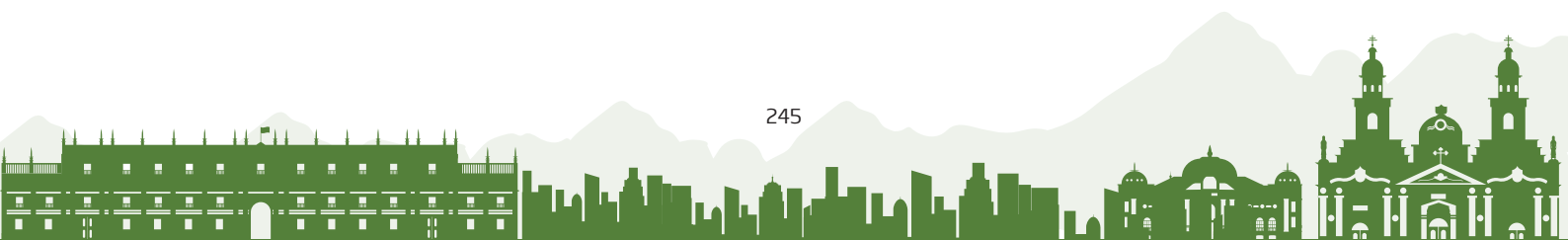
### PP-241

#### Side effects of a pathogenic fungus on stingless bee guarding behaviour

Felipe Chagas Rocha Almeida, José Mauricio Simões Bento, Denise Araujo Alves

Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo, Av. Pádua Dias, 11, Piracicaba, 13418 900, Brazil

Pathogenic fungi have been used worldwide to control crop pests and are assumed to pose negligible threats to the survival of pollinators. Although eusocial stingless bees provide essential pollination services and might be exposed to these biopesticides in tropical agroecosystems, there is a substantial knowledge gap regarding the side effects of fungal pathogens on behavioural traits that are crucial for colony functioning, such as guarding behaviour. Here, we evaluated the effect of *Beauveria bassiana* on the sophisticated kin recognition system of *Tetragonisca angustula*, a bee with morphologically specialized entrance guards. By combining behavioural assays and chemical analyses, we show that guards detect pathogen-exposed nestmates, preventing them from accessing nests. Furthermore, cuticular profiles of pathogen-exposed foragers contained significantly lower amounts of linear alkanes than the unexposed ones. Such chemical cues associated with fungal conidia may potentially trigger aggression towards pathogen-exposed bees, preventing pathogen spread into and among colonies. This is the first demonstration that this highly abundant native bee seems to respond in a much more adaptive way to a potentially infectious threat, outweighing the costs of losing foraging workforce when reducing the chances of fungal pathogen outbreaks within their colonies, than honeybees do.





### PP-242

#### Mortality and morphology of thiamethoxam-tolerant *Apis mellifera* L. after exposure to flupyradifurone

José Washington Santos Oliveira<sup>1</sup>, Gustavo Henrique Simões Pereira<sup>1</sup>, Daiani Rodrigues Moreira<sup>2</sup>, Cinthia Leão Figueira<sup>1</sup>, Douglas Galhardo<sup>1</sup>, Breno Gabriel<sup>3</sup>, Sandra Milena Diaz Puentes<sup>1</sup>, Adriana Aparecida Sinópolis Gigliolli<sup>2</sup>, Maria Claudia Colla Ruvolo Takasusuki<sup>2</sup>, Vagner deAlencar Arnaut de Toledo<sup>1</sup>

<sup>1</sup>Department of Animal Science, State University of Maringá, 87020-900, Maringá, Paraná, Brazil

<sup>2</sup>Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, 87020-900, Maringá, Paraná, Brazil

<sup>3</sup>Department of Exact Sciences, Escola Superior de Agricultura "Luiz de Queiroz" - University of São Paulo, 13418-900, Piracicaba, São Paulo, Brazil

Contamination of bees by pesticides, such as neonicotinoids, is one of the main factors related to colony decline. For this reason, companies have been looking for molecules that promise to be less harmful to bees, including flupyradifurone (FPF). Thus, this work aimed to evaluate the effect of FPF contamination on the mortality and morphology of the midgut of *Apis mellifera* previously selected for thiamethoxam tolerance (tolerant) and unselected (non-tolerant), as well as to evaluate the occurrence of Cross-resistance between neonicotinoid and FPF. For that, worker bees (n=10) were placed in glass flasks containing food contaminated with different concentrations of FPF in the commercial form (Sivanto) and the active ingredient FPF isolated. As a control, bees received only food. The flasks were conditioned in B.O.D at a temperature of 31°C ±3 and humidity 65%±10 for 24h. Five repetitions were performed. After this period, mortality was assessed and the midgut of surviving bees was dissected and processed for histological analysis. It was found that mortality increased simultaneously with the increase in FPF concentration, with bees selected for neonicotinoid tolerance being more sensitive to FPF, especially in the commercial form. The midgut of tolerant and non-tolerant bees showed muscle loosening, digestive cells with changes that suggest the occurrence of cell death and damage to regenerative cells. In the impossibility of epithelial renewal after exposure to FPF, the damage can compromise digestion, leading to nutritional depletion, with damage to other physiological processes and behavior of these pollinators. In view of these results, it was found that thiamethoxam-tolerant bees did not show cross-resistance to FPF, even though these products have structural similarity and in the mode of action, and the evaluated product may be toxic to *Apis mellifera*, depending on the concentrations to which the bees are exposed.

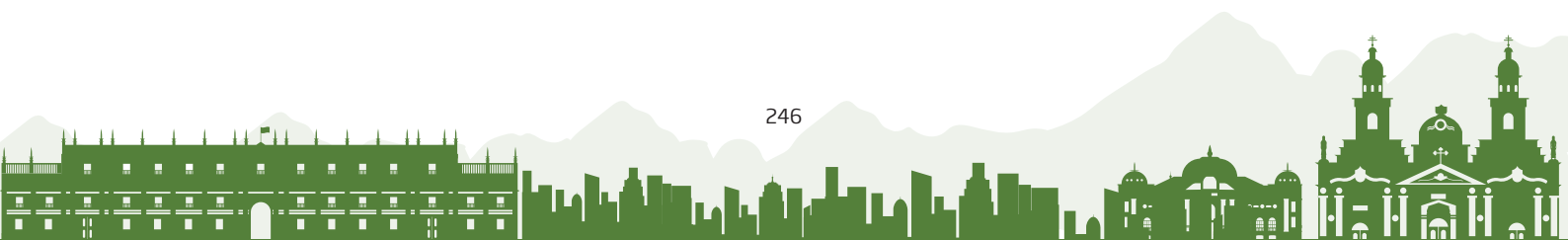
### PP-243

#### Trans-generational immune priming against Chalkbrood (*Ascosphaera apis*) in honeybees

Dalial Freitak, Hanna Bauer, Franziska Dickel

Institute of Biology, Karl-Franzen University of Graz, Graz, Austria

Brood diseases threaten honeybee colonies and are especially hard to treat. Honeybees have sophisticated immune responses against diseases both on the individual, as well as colony level. Especially interesting is that honeybee queens can transfer the immunological signals from their bodies to their offspring, this has been coined as trans-generational immune priming (TGIP). We have demonstrated, that TGIP can be used to develop vaccination against American Foulbrood infection in honeybees. Here we are having closer look how TGIP is functioning in the case of the fungal disease - Chalkbrood. We demonstrate, that oral immunization of the queen leads to upregulation of various immunity related genes, which can be regulated differently in different tissues. We also study the various immune factors in the primed larvae, to better understand which molecules are involved in TGIP in honeybees. We examine the resistance of the larvae against different brood diseases and assess their overall fitness.



### PP-244

#### Impact of honey bee nutrition on the immune system

Belén Branchiccela<sup>1</sup>, Karina Antúnez<sup>2</sup>, Miguel Corona<sup>3</sup>, Sofía Fernández<sup>4</sup>, Pablo Smircich<sup>5</sup>, Ciro Invernizzi<sup>6</sup>

<sup>1</sup>Sección Apicultura, Instituto Nacional de Investigación Agropecuaria. Colonia, Uruguay.

<sup>2</sup>Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay.

<sup>3</sup>Bee Research Laboratory, US Department of Agriculture. Maryland, United States of America.

<sup>4</sup>Plataforma de Salud Animal, Instituto Nacional de Investigación Agropecuaria. Colonia, Uruguay.

<sup>5</sup>Departamento de Genómica, Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay.

<sup>6</sup>Sección Etología, Facultad de Ciencias, Universidad de la República. Montevideo, Uruguay.

During last year's, high honey bee colony losses has been reported worldwide and one of the main factors associated to them is nutritional stress due to agriculture intensification. The immune system is a key factor in bee homeostasis, due to bees relies on the individual (humoral and cellular) and social immunity to defend itself against pest and pathogens. It has been reported that the immune system is costly, and it depends on nutrition. However, in social insects there is not enough evidence supporting this statement. The general objective of this study was to analyze the relationship between honey bee nutrition and the immune system. Honey bee colonies with no access to floral resources were provided with a base diet of canola pollen to ensure colony homeostasis and were divided into three groups according to the diet that they received: sugar syrup (negative control), a synthetic diet based on the amino acids that the bees require (AA) or a diet containing the amino acids and lipids that bees require (AA+L). The transcriptome of heads and abdomens of 7th and 14th days old bees fed with these diets was analyzed by RNA sequencing. Bees fed with AA or AA+L showed overexpression of genes associated with delayed behavioral maturation. The anatomical structure development and its regulation as well as the responses to stimulus were the biological processes overexpressed in well-nourished young bees, but not the immune functions. In conclusions, our results showed that honey bee nutrition affect mainly the expression of genes associated with behavioral maturation rather than to the immunocompetence as a direct effect. However, it is not possible to ruled out that a better immune response can be display by better nourished bees challenged with pathogens.

### PP-245

#### Mitigating Pesticide-Related Detrimental Effects on Honeybees with Polyphenolic Compounds

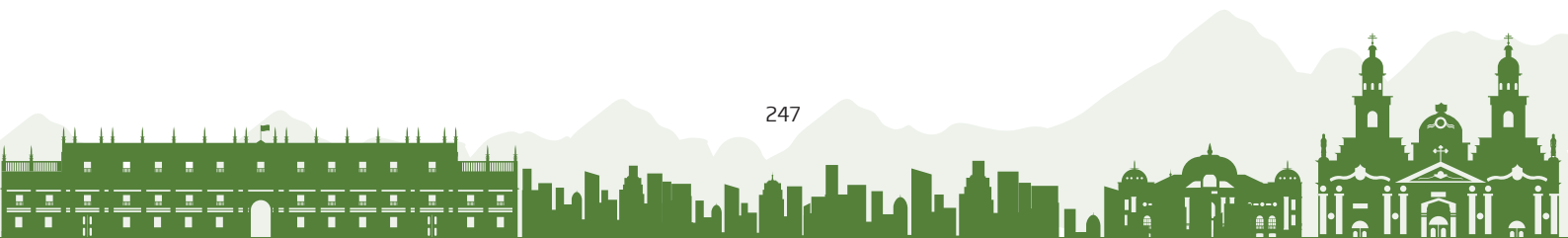
Saeed Mohamadzade Namin<sup>1</sup>, Tekalign Begna<sup>1</sup>, Youngrak Kang<sup>2</sup>, Daniel Bisrat<sup>3</sup>, Delgermaa Ulziibayar<sup>2</sup>, Arezoo Najarpour<sup>2</sup>, Chuleui Jung<sup>2</sup>

<sup>1</sup>Agriculture Science and Technology Research Institute, Andong National University, Republic of Korea

<sup>2</sup>Department of Plant Medicals, Andong National University, Republic of Korea

<sup>3</sup>Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Honeybees are crucial for pollinating two third of the world's crop species. Honeybee decline has become a significant threat to global food production. One of the factors of honey bee decline is the exposure to pesticides, while foraging. Curcumin (CU) and rosmarinic acid (RA) are natural polyphenolic compounds found in turmeric and rosemary, respectively, known for their antioxidant, anti-inflammatory, and pro-apoptotic properties, and have been used in traditional medicine. In our study, we investigated the effects of pre-feeding and post-feeding to honeybees with different concentrations of CU and RA (50, 100, and 200 ppm) on reducing the mortality of worker bees (nurses and foragers) exposed to lethal doses of acetamiprid, carbaryl, and flupyradifurone. Results showed that only CU100 was effective in reducing the mortality of nurse bees in both pre-feeding and post-feeding experiments when exposed to acetamiprid, while RA100 only decreased the mortality of foragers in the pre-feeding test. For bees exposed to carbaryl, RA200, and CU100 significantly reduced the mortality of both nurses and foragers in the post-feeding test, but were not effective in the pre-feeding test. In bees exposed to flupyradifurone, CU100 was the only effective concentration in mitigating the mortality of nurses and foragers in both pre-feeding and post-feeding tests, with RA50 only reducing the negative effects of the pesticide on nurse bees. Our results also demonstrated that CU and RA had a positive effect on the longevity of honeybees in laboratory conditions, with the highest longevity observed in bees that received RA50 and RA100 for 48 hours. Gene expression analysis 24 hours after treatment showed that feeding CU upregulated genes related to detoxification capacity, while the detoxification mechanism of RA remains unclear and further transcriptome analysis may be required further. Our findings suggest that the use of polyphenolic compounds; such as CU and RA may help reduce the pesticide risk, and increase their lifespan, potentially leading to improved honey bee health.



### PP-246

#### Evaluation of the nutritional and sanitary status and the presence of agrochemicals in *Apis mellifera* hives (Hymenoptera: Apidae) exposed to agricultural intensification

Pablo J Moja<sup>1</sup>, Ivana N Macri<sup>2</sup>, Marina Basualdo<sup>4</sup>, Diego Cristos<sup>5</sup>, Walter M Farina<sup>3</sup>

<sup>1</sup>EAA Cuenca del Salado. INTA, Rauch, Buenos Aires, Argentina, Laboratorio de Insectos Sociales, DBBE-FCEN-UBA, Buenos Aires, Argentina, IFIByNE (UBA-CONICET), Buenos Aires, Argentina

<sup>2</sup>Laboratorio de Insectos Sociales, DBBE-FCEN-UBA, Buenos Aires, Argentina, IFIByNE (UBA-CONICET), Buenos Aires, Argentina, Instituto de Ingeniería Rural, Centro de Investigación de Agroindustria (CIA), INTA, Hurlingham, Buenos Aires, Argentina.

<sup>3</sup>Laboratorio de Insectos Sociales, DBBE-FCEN-UBA, Buenos Aires, Argentina, IFIByNE (UBA-CONICET), Buenos Aires, Argentina,

<sup>4</sup>PROANVET-Facultad de Ciencias Veterinarias. UNICEN, Buenos Aires, Argentina

<sup>5</sup>Instituto Tecnología de los Alimentos, Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Buenos Aires, Argentina.

The nutritional, health and chemical contamination status of *Apis mellifera* bee colonies depends on the nectar supply in their environments, which is declining as intensification of land use and the use of agrochemicals increases. La Cuenca del Rio Salado (Buenos Aires Province) in Argentina provides an ideal environment in which to study the variables and their impact. The present work has monitored colonies located in two contrasting environments that vary according to the degree of agricultural disturbance: one area is exposed to modern agricultural practices and the other less affected. Beehive colonies in both environments have received acaricide treatment and protein supplementation. During autumn 2018, the condition of the colonies in both areas was assessed by determining their nutritional status, health and population profiles as well as the presence of agrochemicals in their honey, beeswax and bee bread. Principal component analysis (PCA) identified the environment having the greatest agricultural impact as hosting colonies with a higher concentration of glyphosate in their wax and a higher concentration of glufosinate in their honey. These colonies also had higher levels of varroa infestation. In the environment less impacted by agricultural disturbance, a higher protein concentration in the hemolymph of the bees was found. This work is a first step towards the identification of colony responses to differing environments, health treatments, and protein supplementation in the agricultural-livestock region of the La Cuenca del Rio Salado.

### PP-247

#### Brazilian Bee Observatory: program to mitigate losses of bee colonies

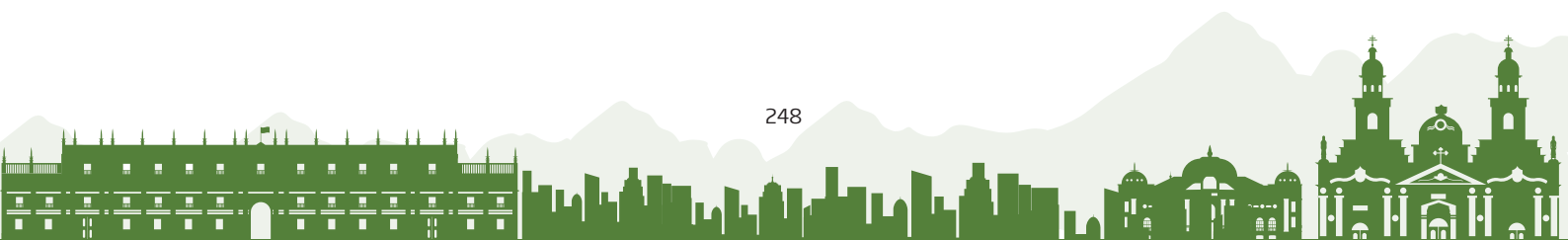
Betina Blochtein<sup>1</sup>, Jenifer Dias Ramos<sup>2</sup>, Luciana Abrego<sup>3</sup>, Cristiano Menezes<sup>2</sup>

<sup>1</sup>Mais Abelhas Consultoria Ambiental

<sup>2</sup>Embrapa Meio Ambiente – Jaguariuna/SP

<sup>3</sup>Ministério da Agricultura e Pecuária

Bees are essential for maintaining the pollination ecosystem service, necessary for biodiversity conservation and agricultural growth. However, the decline of pollinators on a global scale became part of the 2030 agenda, based on the inclusion of pollinators in the Sustainable Development Goals established by the UN. In Brazil, bee deaths in different states indicate the need to identify the causes of incidents, as well as to link this information in a systematic way on an official public platform. In this context the Brazilian Bee Observatory Program, coordinated by EMBRAPA Environment (Jaguariuna, SP, Brazil) together with the Ministério da Agricultura e Pecuária, in collaboration with public and private agents, has the mission of supporting actions to mitigate bee deaths in the country, and OBJECTIVES: 1) Strengthen the systematization of assistance with bee deaths by public agents; 2) Consolidate the records in an Official Data Integration Platform (POD), with the possibility of establishing a causal link with the agents that caused the incidents; 3) Support laboratories dedicated to animal health and detection of pesticides in bees; 4) Support training actions in good agricultural and beekeeping practices, and dialogue between those involved in the theme; 5) Stimulate the registration of apiaries/meliponary to official institutions; 6) Support the development of public policies based on POD data for the protection of pollinators. The implementation of the Observatory started in August/2022, and the first phase included the diagnosis regarding the resources to deal with incidents with bees with the assistance network of Brazilian states (RS, SC, PR, SP, MS, MT, GO). The second phase, currently underway, is the construction of the POD, an application for use in the field with forms on bee mortality, georeferenced data, and an interface with WebGis for disseminating the data on dashboards. The subsequent phases will contemplate the implementation of the POD, training of human resources and discussions with different sectors of society aiming to mitigate the deaths of bees in Brazil. The initial steps of the Program inspire optimism, and advances are dynamic and dependent on official data records in the POD.



### PP-248

#### Degradation of pesticides during bee bread fermentation

Louisa Hooven<sup>1</sup>, Nina Rudin<sup>3</sup>, Jeff Morre<sup>2</sup>, Claudia Maier<sup>2</sup>

<sup>1</sup>Department of Horticulture, Oregon State University, Corvallis, Oregon, USA

<sup>2</sup>Department of Chemistry, Oregon State University, Corvallis OR, USA

<sup>3</sup>Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, USA

Environmental degradation influences the persistence and risk of residual pesticides. However, once honey bees bring pesticide-laden pollen into the hive, new factors are at play. In the warm, dark, hive, photolysis no longer occurs, and pH changes as microbial communities ferment pollen into moist bee bread. These bacteria and yeasts have their own complement of xenobiotic-metabolizing enzymes, which may act to break down susceptible pesticides. Toxic pesticides which degrade quickly in bee bread may be difficult to link to acute bee kills, while those that resist microbial degradation may pose a chronic risk to hive health. Currently, there are no test methods to determine whether a given pesticide breaks down immediately or persists in bee bread, which is needed for understanding risk to pollinators which consume stored pollen as a protein source for larvae. We have developed a simple laboratory assay to replicate pollen fermentation into bee bread. Chemical compounds of interest can be incorporated into pollen at appropriate concentrations, sampled at intervals and submitted for chemical analysis, providing an estimate of how quickly a given pesticide breaks down during bee bread fermentation. This assay also allows us to investigate the impact of additional variables such as pesticide mixtures, synergists, and formulations on the persistence of any given pesticide. Many pesticide formulations extend product efficacy by protecting active ingredients from environmental degradation, but also potentially prolong their exposure time to honey bees. Chlorothalonil is a fungicide which has been associated with adverse effects on honey bee larvae. Our initial work suggests that chlorothalonil degrades more quickly when the active ingredient alone is incorporated in pollen compared to when it is associated with the formulated product Bravo. Bee bread fermentation is highly variable, and our assay is an imperfect replica of this biological process. However, it will allow direct and standardized comparison of degradation between chemical substances, which can inform pollinator risk assessment. Use of our assay could enable the selection or modification of pesticide products and formulations which are less persistent in hive foodstuffs and more protective of pollinator health.

### PP-249

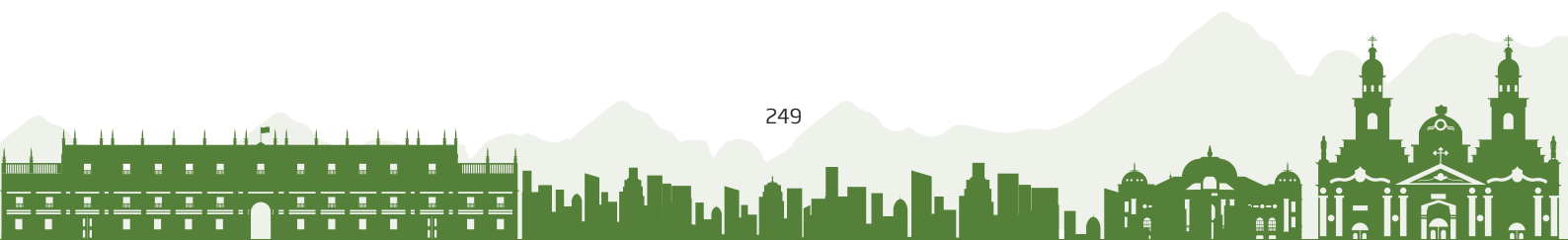
#### Evaluation of *Ganoderma australe* (Fr.) Pat. extracts for the control of nosemosis (*Nosema* spp.) in bee hives (*Apis mellifera* L.) in the Choroico sector, La Unión, Los Ríos Region

Richard Inostroza<sup>1</sup>, Esteban Basoalto<sup>1</sup>, Leticia Silvestre<sup>1</sup>, Sigisfredo Garnica<sup>2</sup>

<sup>1</sup>Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Valdivia, Chile.

<sup>2</sup>Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.

*Apis mellifera* L. is considered the most important pollinating agent, but in recent years, the mortality of this insect has been increasing worldwide. There are different causes that would be affecting bees, and one of the most important is the microsporidium *Nosema* spp., a difficult treat disease because its only control tool, the antibiotic Fumagillin, is prohibited internationally. This situation promotes the search for new methods to control this disease, based on less harmful compounds and ideally of natural origin. Consequently, the use of mushroom extracts is especially attractive. Due to the antimicrobial and antifungal capacities of *Ganoderma* genus species, it was suggested that treatment with extracts of the *Ganoderma australe* fungus would reduce the incidence of nosemosis (*Nosema* spp.) in *Apis mellifera* bee hives. For this, powdered (lyophilized) extracts of *G. australe* were applied, at a concentration of 0.5%, mixed with a maintenance/stimulus syrup. The treatments were applied on two different dates, corresponding to January-February and May, respectively. In both treatments, significant differences were observed in the number of spores, respecting to the initial amount present in the hives. After 2 days post-application of the syrup with the fungus extract, compared to control hives, which received only syrup. Therefore, application of *Ganoderma australe* extracts, in syrup, allows to reduce the incidence of *Nosema* spp. in bee hives.



### PP-250

#### Behavioral alteration in bees infected with deformed wing virus variant A (DWV-A) to the volatile fraction of different types of pollens

Diego Eduardo Silva Vasquez<sup>1</sup>, Marisol Vargas<sup>1</sup>, Ricardo Ceballos<sup>2</sup>, Juan Alveal<sup>2</sup>, Nolberto Arismendi<sup>3</sup>, Nelson Zapata<sup>1</sup>

<sup>1</sup>Laboratorios de Virología y Sanidad Apícola, Facultad de Agronomía, Universidad de Concepción, Chillán, Chile.

<sup>2</sup>Instituto de Investigaciones Agropecuarias, INIA Quilamapu, Chillán, Chile.

<sup>3</sup>Centro de Investigación Austral Biotech, Facultad de Ciencias, Universidad Santo Tomás, Valdivia, Chile

Pollen is crucial in the bee nutrition. From the pollens, bees obtain proteins, lipids and some minerals that help them in their physiological development. It has been shown that sick bees have a preference for pollen, which is related to social immunity behaviors by searching for foods with beneficial components for their health. However, there is no evidence of this behavior in bees infected with deformed wing virus (DWV), a pathogen that can affect olfactory perception and gene expression of different olfactory-related proteins. Given that, the aim of this study was to evaluate the behavioral preference of bees infected with deformed wing virus variant A (DWV-A) to pollen scent. For this purpose, different types of pollen were collected using traps placed in the entrance of the hives: multifloral (1), monofloral from blackberry (2), monofloral from mustard (3) and monofloral pollen from native ulmo tree (4). The volatile fraction was collected from each pollen for 24 h using dynamic headspace equipment. Then, newly emerged bees were individually inoculated with a viral suspension ( $1 \times 10^9$  viral copy number per bee), using uninoculated bees as a control ( $1 \times 10^2$  viral copy number per bee), 15 days post inoculation, the bees were exposed to the volatile fraction of each pollen, using a Y-type olfactometer. For each stimulus, 40 bees were used for each treatment, considering each bee as a replicate of the trial. The viral load of each bee was quantified by qPCR at the end of the bioassay. The DWV-A load was on average 68% higher in inoculated bees compared to non-inoculated bees. The DWV-A infected bees showed a lower preference for all type of pollens tested, being a 30, 37, 40 and 39% for pollen 1, pollen 2, pollen 3 and pollen 4, respectively. The results suggest that DWV-A could affect the behavioral preference of honey bees for different food sources, which ruling out possible routes of self-medication, at least in the processes of preference of floral sources rich in pollens and when the bees are infected with DWV.

### PP-251

#### Antiviral activity of pollen-derived terpenes and their effect on the survival of honey bees infected with variant A of the deformed wing virus

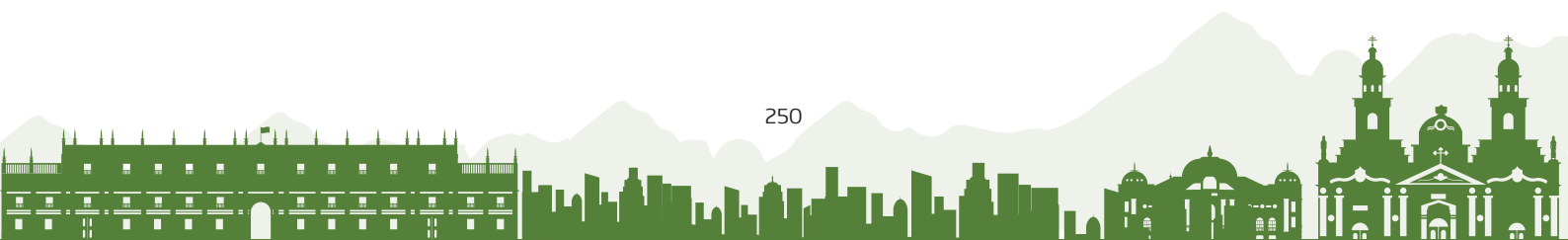
Diego Eduardo Silva Vasquez<sup>1</sup>, Felipe Becerra<sup>1</sup>, Marisol Vargas<sup>1</sup>, Sandra Vargas<sup>1</sup>, Nolberto Arismendi<sup>2</sup>, Nelson Zapata<sup>3</sup>

<sup>1</sup>Laboratorios de Virología y Sanidad Apícola, Facultad de Agronomía, Universidad de Concepción, Chillán, Chile.

<sup>2</sup>Centro de Investigación Austral Biotech, Facultad de Ciencias, Universidad Santo Tomás, Valdivia, Chile

<sup>3</sup>Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Concepción, Chillán, Chile.

Pollen and nectar are rich in various phytochemicals including phenols, alkaloids, and terpenes. The latter are the largest and most diverse group of natural compounds. Pollen intake influences the physiological metabolism of the bees and its tolerance to pathogens. Previous studies have shown the antiviral activity of monoterpenes, which makes them attractive for evaluate their activity against the infection generated by the deformed wing virus (DWV) in bees. For this reason, the aim of the present study was to evaluate the effect of three terpenes previously reported in different pollens, on the survival and antiviral activity of honey bees (*Apis mellifera*) infected with variant A of the deformed wing virus (DWV-A). Thus, newly emerged bees were individually infected with a suspension of DWV-A ( $1 \times 10^9$  number of genomic copies per bee), locked in cages ( $n=60$ ) and arranged in a bioclimatic chamber under controlled conditions (30 °C temperature, 60–70% relative humidity). Then, every five days and for 48 hours, they were fed ad libitum with 16 ppm of the different terpenes (treatments): Limonene, 1-4 cineole, linalool, prepared in 60% sugar syrup. Four repetitions per treatment were established. The survival was evaluated daily for 20 days post viral inoculation (dpi), and the same time, five bees from each replicate per treatment were collected every five days to determine viral load. The results showed that at 20 dpi, the bee survival was 85, 71 and 80% when the bees were fed with Limonene, 1-4 cineol and linalool, respectively. On the other hand, bees inoculated with virus and not treated with terpenes (control), the survival was 52%. The viral load decreased at 30%, 42% and 37% when the DWV-A infected bees were fed with limonene, 1-4 cineol and linalool, respectively; and when were compared with not treated infected bees. The results of this study showed the antiviral activity of different terpenes commonly found in pollens and their benefit impact in the survival of bees infected with DWV-A.





### PP-252

#### Exposure to the fungicide bixafen and food availability alter the expression of immune system genes in honey bees

Aline Yukari Kato<sup>1</sup>, Cássia Regina de Avelar Gomes<sup>1</sup>, Matheus Franco Trivellato<sup>1</sup>, Simone Beneti<sup>3</sup>, Tainá Angelica De Lima Freitas<sup>1</sup>, Thaís Regina Ramos Alves<sup>1</sup>, Yara Martins Molina Ferraz<sup>1</sup>, Jaqueline Biller<sup>2</sup>, Daniel Nicodemo<sup>2</sup>

<sup>1</sup>Post Graduate Program in Animal Science, Sao Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil

<sup>2</sup>Department of Animal Science, Sao Paulo State University (Unesp), College of Agricultural and Technological Sciences, Dracena, Brazil

<sup>3</sup>Post Graduate Program in Technology and Animal Science, Sao Paulo State University (Unesp), College of Agricultural Technological Sciences, Dracena, Brazil

Bees are susceptible to several environmental stressors and great emphasis has been given to the harmful effects of pesticides, including fungicides, for which there is little or no use restriction related to the environmental safety of these beneficial insects. Health risks to bees inherent to the effects of pesticides may be greater when the nutritional status of colonies is compromised due to food shortages. The objective of this work was to evaluate whether honey bees from colonies submitted to two feeding managements present differences regarding the expression of immune system genes when exposed to the fungicide bixafen. The diet of a group of colonies was supplemented with syrup based on water and sugar in equal proportions and a protein paste obtained from a mixture of four parts of pollen and one part of honey for 15 weeks. For the same period of time another group of colonies did not receive food supplements and had their natural pollen consumption restricted through the use of pollen collectors. Then, forager bees were collected from the entrance of the hives and anesthetized in a freezer. Bees were exposed by contact to the fungicide bixafen with doses of 1 or 7 µg diluted in acetone. In bees of the control group, only acetone was applied. After 48 hours of exposure to the fungicide, the bees were anesthetized and dissected, using the abdomen (fat body plus carcass, free from intestine) to evaluate the expression of genes abaecin, apidaecin defensin and hymenoptaecin using realtime qPCR. Feeding treatment did not result in differentiated gene expression when bees were not exposed to bixafen. When exposed to the higher dose of the fungicide, there was an increase in the expression of all genes studied, except for abaecin and hymenoptaecin in bees from colonies with restricted feeding. The expression of apidaecin and hymenoptaecin genes was higher in bees from hives with restricted feeding compared to those from hives that received food supplements. Well-nourished bees may have a more efficient immune response to the challenge resulting from exposure to the fungicide bixafen.

### PP-253

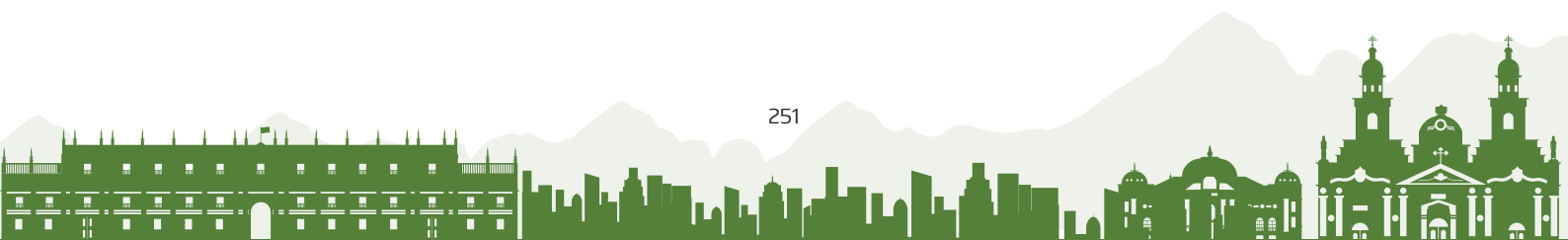
#### Application of thymus vulgaris L in the treatment of Nosema sp. In honey bee colonies in Algeria (Results of the PRIMA-SafeAgroBee project)

Noureddine Adjlane<sup>1</sup>, Yamina Haider<sup>1</sup>, Fani Hatjina<sup>2</sup>

<sup>1</sup>Department of Agronomy, University of Boumerdes, Algeria

<sup>2</sup>Division of Beekeeping, Inst. of Animal Science, ELGO 'DIMITRA', Greece

Despite all the efforts made by beekeepers, a winter loss of hives of around 10 to 15% is generally observed in Algeria. One of these agents of hive loss is *Nosema* spp which is known only very little. Nosemosis is a parasitic disease of the bee caused by a parasite of the fungidea class (formerly classified in the protozoa). This pathology affects the three castes of bees in the hive and is due to the proliferation of the parasite *Nosema apis* or *ceranae* in the intestinal cells of its hosts. The aim is to know whether, under field conditions, thymol administered as a preventive measure can reduce the count of spores in the digestive system of bees and thus improve their state of health during the wintering and post-wintering period. The goal is to administer the oil in the feeding syrup in the fall in order to limit the multiplication of the agent of nosemosis during wintering and spring. This work was carried out as part of a PRIMA (SAFEAGROBEE) project on the preservation of the honey bee. The study was carried out by a beekeeper in an apiary divided into 2 experimental batches of 30 hives contaminated with *Nosema* spores, whether or not they received a thymol supplement in the feeding syrup. The results obtained demonstrated a negative effect of thymol on colony development with an increase in mortalities when comparing the two batches. This molecule also had some effectiveness in reducing the incidence of nosemosis in the colonies. The mode of action of thymol remains to be defined, but it would affect cell permeability and that of mitochondria or even intervene in the process of sporulation of *Nosema*. Other tests are to be carried out in order to confirm the results and to disseminate the methods to beekeepers.



### PP-254

#### Stonebrood. An emerging disease for Argentine wild bees?

Ailen Alejandra Solis<sup>1</sup>, Leopoldo Álvarez<sup>2</sup>, Marco Antonio Tizzano<sup>1</sup>, Romina Della Vedova<sup>1</sup>, Mariano Lucia<sup>2</sup>, Francisco José Reynaldi<sup>3</sup>

<sup>1</sup>Área Micología, Centro de Microbiología Básica y Aplicada (CEMIBA). Facultad de Ciencias Veterinarias. Universidad Nacional de La Plata (UNLP). La Plata, Prov. Buenos Aires, Argentina.

<sup>2</sup>División Entomología. Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (UNLP). La Plata, Prov. Buenos Aires, Argentina.

<sup>3</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

Bees were the most important group of pollinators worldwide, but in the last fifteen year their decline has become a growing problem. Several factors, such as habitat loss, pesticides, climate change, parasites and diseases and their interactions were the most important ones. In Argentina very few information is available. Related to bee pathogens that affect wild bees, only sporadic reports were available in the last years, but no reports of stonebrood (*Aspergillus flavus*) affecting bees were informed. As fungi are the aetiological agents that are less frequently reported, they receive very little attention and are understudied in scientific research.. Moreover, (*A. flavus*) compared to other fungal diseases such as chalkbrood caused by (*Ascosphaera apis*), it is considered of minor importance in apiculture. However, this fungus is the second most reported cause of human aspergillosis. We study twenty-six brood cells nest of a nest aggregation of (*Melitoma segmentaria*) and (*Ancylloscelis halictoides*) located in a vertical clay bank located on artificial hill of approximately 1m high and an area of 16m<sup>2</sup> in Berisso, Buenos Aires (-34.8607, -57.8756). Brood cells dead were opened, and all samples were processed in order to eliminate any possible superficial fungal agents. Later, samples were cut in pieces and cultured on YGPSA (yeast extract 10 g, glucose 10 g, KH<sub>2</sub>PO<sub>4</sub> 13.5 g, soluble starch 10 g, and agar 20) for 21 days at 37 °C in darkness. We isolated in fourteen samples (*A. flavus*), identify by macromorphological, micromorphological, PCR assays, sequencing and BLAST identification. (*A. flavus*) have been reported, sporadically, affecting honeybee of Argentina. To our knowledge this is the first report of (*A. flavus*) affecting wild bees in Argentina.

### PP-257

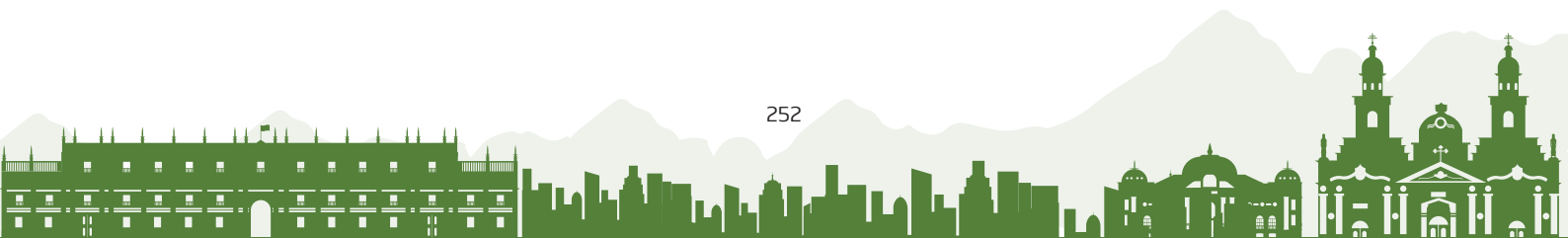
#### Toxicity of *Spathodea campanulata* nectar to *Scaptotrigona bipunctata* Lepelletier, 1836 (Hymenoptera: Apidae)

Eloísa Magalhães Pereira<sup>1</sup>, João Marcos Figueiredo Matano<sup>1</sup>, Maria Carolina Paleari Varjão De Oliveira<sup>1</sup>, Wingly Santos Beltrame<sup>1</sup>, Daiani Rodrigues Moreira<sup>1</sup>, Isac De Macedo Da Penha<sup>2</sup>, Adriana A.Sinópolis Gigliolli<sup>1</sup>, Maria Claudia Colla Ruvolo-Takasusuki<sup>1</sup>

<sup>1</sup>Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, Paraná, Brazil

<sup>2</sup>Department of Animal Science, State University of Maringá, Paraná, Brazil

Stingless bees are essential for maintaining ecosystems, biodiversity and agriculture. They collect nectar and pollen from flowers as food, however, exotic and native species can produce substances that can intoxicate bees when they come into contact with these resources. Among these species, *Spathodea campamulata* stands out, an African Bignoneaceae, introduced in Brazil for ornamental purposes. Therefore, the objective of this work was to evaluate the effect of *S. campanulata* nectar on mortality, alteration in the chromatin structure and morphology of the midgut of *Scaptotrigona bipunctata*. For this purpose, adult workers were collected and contaminated by oral exposure with different concentrations of *S. campamulata* nectar for 24, 48 and 72 hours. As control, candy was provided as food. Then, the midgut of bees exposed to nectar and control were dissected, subjected to cytochemical analysis to determine the critical concentration of electrolytes (CEC), as well as morphological analysis. After exposure, it was found that the highest mortality rate was obtained at higher doses and longer exposure times, with all individuals dying within 24 hours at 90% and 100% nectar concentrations. In the midgut, CEC indicated chromatin compaction, indicative of reduced gene expression. In addition, it was possible to observe muscle loosening resulting in disorganization and degeneration of the epithelium, presence of cytoplasmic vacuolization and apical cytoplasmic protrusions in digestive cells, as well as changes in regenerative cells. It can be verified that the nectar of *S. campanulata* was toxic for *S. bipunctata*. Even at sublethal concentrations, the damage caused to the midgut can compromise other physiological processes essential for the survival of these pollinators. Thus, toxicity studies of substances produced by plants are essential, as they can interfere with the maintenance of colonies if bees collect their floral resources as food. us, toxicity studies of substances produced by plants are essential, as they can interfere with the maintenance of colonies if bees collect their floral resources as food.



### PP-258

## Development of a real-time multiplex RT-PCR to differentiate between Deformed Wing Virus (DWV) subtypes

Fernanda Noemi Gonzalez<sup>1</sup>, Matias Moragas<sup>3</sup>, Cecilia Ferrufino<sup>1</sup>, Graciela Rodriguez<sup>2</sup>, María Jose Dus Santos<sup>1</sup>

<sup>1</sup>Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Virología. Unidad Ejecutora IVIT INTA-CONICET.

<sup>2</sup>Instituto Nacional de Tecnología Agropecuaria (INTA), E.E.A. Hilario Ascasubi.

<sup>3</sup>Laboratorio de Biología celular y retrovirus. Servicio de Microbiología, Hospital de Pediatría "Prof. Dr. Juan P. Garrahan"

Deformed wing virus (DWV) is a highly prevalent pathogen of concern for honeybee health, transmitted by the Varroa destructor mite. There are three DWV variants -DWV-A, DWV-B, DWV-C and their accurate and rapid identification is crucial for disease control measures. The presence of the virus has been reported in Argentina, but the DWV-A and DWVB variants have recently been characterized by sequencing. For this reason, the objective of this work was to develop a multiplex RT-qPCR to differentiate the DWV-A from DWV-B/C variants. We designed a unique set of primers that amplify a conserved 180 bp fragment within the helicase gene. Also, two labeled probes were designed, one specific for DWV-A and the other for DWV-B/C. Positives bee samples (also a plasmid containing the target sequence) and a synthetic gene (ultramers) were used as positives controls for DWV-A and DWV-B/C, respectively. The dynamic range, detection limit, and precision of the technique were determined according to the MIQE guidelines. The efficiency was calculated with a standard curve, which was prepared using known concentrations of each control ( $2.3 \times 10^7$  to  $2.3 \times 10^2$  gene copies/ $\mu$ l); efficiency values were 99% for the DWV-A probe and 89.6% for the probe DWVB/C. The reaction mix in each assay consisted of 800 nM of each primer and 200 nM of each specific probe in a final volume of 25  $\mu$ L. In addition, DWV positive and negative samples previously identified in our laboratory were used to assess the specificity of the technique. The developed RT-qPCR assay is sensitive, rapid, and allows accurate identification of DWV-A and DWV-B/C variants. Our findings suggest that this technique can be used to monitor colonies and evaluate DWV variants in different ecoregions of the country.

### PP-259

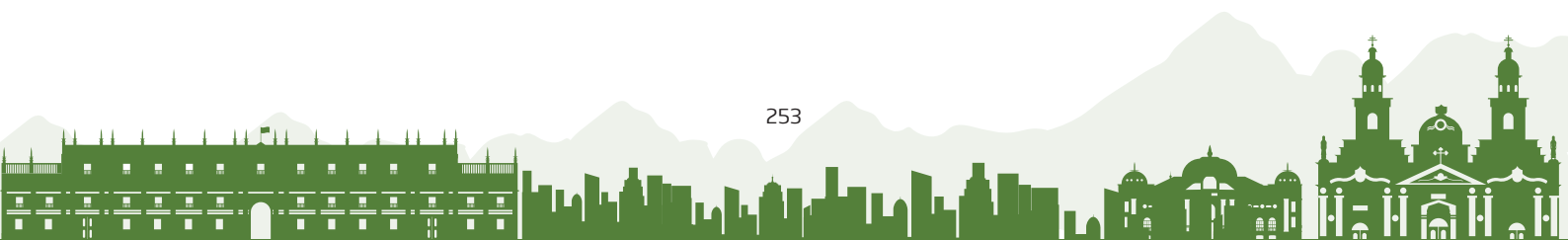
## Imidacloprid Impair The Midgut Of Bees *Apis Mellifera* L. Neonicotinoid Thiamethoxam Tolerant

Maria Carolina Paleari Varjão De Oliveira<sup>1</sup>, João Marcos Figueiredo Matano<sup>1</sup>, Eloísa Magalhães Pereira<sup>1</sup>, Viviana Aparecida Mendes<sup>1</sup>, Daiani Rodrigues Moreira<sup>1</sup>, Isac De Macedo da Penha<sup>2</sup>, Vagner De Alencar Arnaut de Toledo<sup>2</sup>, Adriana Aparecida Sinópolis Giglioli<sup>1</sup>, Maria Claudia Colla Ruvolo-Takasusuki<sup>1</sup>

<sup>1</sup>Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, Paraná, Brazil

<sup>2</sup>Department of Animal Science, State University of Maringá, Paraná, Brazil

*Apis mellifera* bees are raised on a large scale for the production of honey, wax, propolis, royal jelly, among other products. These social insects also perform the ecosystem service of pollination, which is essential for the reproduction of flowering plants. Currently, there is a great dependence on agrochemicals for crop management and pest control in agriculture, which can directly or indirectly compromise pollinators. In this sense, this study aimed to analyze the effects of the neonicotinoid imidacloprid on the midgut of *A. mellifera* worker bees previously selected for tolerance to the neonicotinoid thiamethoxam (tolerant). The bioassays were carried out with *A. mellifera* workers tolerant and non-tolerant to thiamethoxam, exposed orally to the insecticide imidacloprid through syrup. The exposure took place during periods of 24, 48 and 72 hours, in which, after exposure to imidacloprid, the bees were anesthetized with cold and dissected to extract the midgut for morphological analysis. After the treatments, imidacloprid promoted externally in the midgut disorganization, loosening and flattening in the longitudinal muscle fibers. In the circular musculature thinner fibers and their approximation. In the periods of 24 and 48 hours, internally, there was epithelial disorganization with changes in the format of the digestive cells, formation of pyknotic nuclei and cell loss into the lumen. Additionally, in 72 hours there was degradation of the digestive cells, in addition to the formation of secretion and deformation in the brush border. In this way, the insecticide imidacloprid compromised cellular structures essential for the functioning of the bee's intestine, which even in sublethal concentrations can affect the survival of these insects. Thus, it is essential that toxicological tests directed to bees are carried out in order to verify how these compounds can affect pollinators to help in the development of less aggressive molecules.



### PP-260

#### First report of small hive beetle (SHB), “*Aethina tumida*”, in Rio Grande do Sul state, southern Brazil

Fernanda Noemi Gonzalez<sup>1</sup>, Matias Moragas<sup>3</sup>, Cecilia Ferrufino<sup>1</sup>, Graciela Rodriguez<sup>2</sup>, María Jose Dus Santos<sup>1</sup>

<sup>1</sup>Programa de Sanidade Apícola (PNSAp), Departamento de Vigilância e Defesa Sanitária Animal (DDA), Secretaria da Agricultura, Pecuária, Produção Sustentável e Irrigação (SEAPI) do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

<sup>2</sup>Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF), Departamento de Diagnóstico e Pesquisa Agropecuária (DDPA), Eldorado do Sul, Rio Grande do Sul, Brazil

<sup>3</sup>Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil

<sup>4</sup>Laboratório Federal de Defesa Agropecuária em Goiás (LFDA-GO), Ministério da Agricultura e Pecuária (MAPA), Goiânia, Goiás, Brazil

Brazil is one of the largest producers and exporters of honey in the world, and beekeeping activity has been growing in recent years. The Rio Grande do Sul state (RS) is the main honey producer in the country. The occurrence of diseases concerns health authorities. Therefore, from the first report of the occurrence of the Small Hive Beetle (SHB), *Aethina tumida* (Coleoptera: Nitidulidae) in Brazil in 2016 in Piracicaba - SP, the health authorities of RS increased the level of attention on the subject, for be a mandatory reporting case in Brazil. The aim of this work is to report the first record of *Aethina tumida* in RS state, Southern Brazil. On August 11, 2022, the official veterinary service of the Secretary of Agriculture, Livestock and Rural Development (SEAPDR) of RS was informed about the suspected occurrence of SHB in a farm in Triunfo municipality. An on-site inspection was carried out, the beekeeper had 60 hives, reported having lost at least another 60 due to management errors reported by him. He informed having purchased used boxes and materials from the state of São Paulo (SP) in poor condition. During the inspection, veterinarians from SEAPDR examined six hives, in four of which they found beetles with morphological characteristics compatible with the description of *A. tumida*. Beetle samples were placed in vials containing 70% ethanol for diagnosis. At this time, the farm was closed, and the entry or exit of hives was not allowed. The samples were sent to the Federal Agricultural Defense Laboratory (LFDA/MAPA), in Goiás. The identification of the beetle species was done using sequencing of a fragment of the COI gene of 709 base pairs and the identity found was 98.31% compared to *Aethina tumida* sequences available in GenBank (NCBI). The beekeeper was immediately informed about the confirmation of SHB infestation on his farm, and he was instructed to follow technical recommendations for controlling the infestation. This work confirms the diagnosis of the first case of *Aethina tumida* in RS state, expanding the geographic distribution of this parasite and alerting health authorities about measures to control the disease.

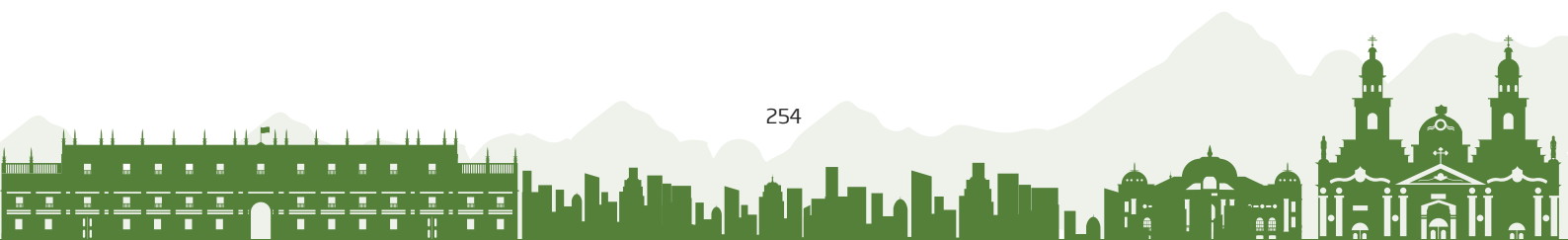
### PP-261

#### Selection and improvement of honey bees in Cuba

Adolfo Pérez Piñeiro, Alejandro Pérez Morfi, Carlos Ariel Yadró, Anais Rodriguez, Carlos Enrique Rodríguez Rosell

Centro de Investigaciones Apícolas, La Habana, Cuba

Since the 1980s, Cuba has undertaken two honey bee selection programs aimed at improving honey productivity and disease resistance. The first program was in effect until the 1990s, while the current program began in 2012. The process consists of a massive selection, in which colonies of beekeepers from six provinces are chosen for their productivity and absence of disease symptoms. These colonies are then placed in selection apiaries, where their honey production and hygienic behavior are assessed over six months. From this population, colonies with the highest yields are selected and certified as breeding stock for the next generation of commercial colonies, which serves as the foundation for the subsequent selection generation. The breeding colonies are reproduced in certified queen honey bees rearing centers where free mating is used, but the area is saturated with drones from the top-performing certified breeding stock colonies. To date, 1408 colonies have been certified as breeding stock from the inception of this program to the current sixth selection generation. In the latest selection cycle, the breeding stock attained a productive yield of  $46.45 \pm 16.32$  kg of honey/colony (mean  $\pm$  SD) in six months, hygienic behavior of  $57.35 \pm 8.50\%$  in eight hours, a VSH greater than 80% and a *Varroa* infestation rate on adult bees of  $4.5 \pm 2.7\%$ . The program has positively impacted beekeeping nationally, raising the average yield per colony from 41.94 kg (2000-2013) to 44.96 kg (2014-2023).





### PP-262

#### Acute bee paralysis virus infection and mortality can be reduced by RNA interference oral administration in adult Honey bees *Apis mellifera*

Cecilia Ferrufino<sup>1</sup>, Alejandra Scannapieco<sup>2</sup>, Romina Russo<sup>1</sup>, Fernanda Gonzalez<sup>1</sup>, Ricardo Salvador<sup>1</sup>, María José Dus Santos<sup>3</sup>

<sup>1</sup>Instituto Nacional de Tecnología Agropecuaria

<sup>2</sup>Consejo Nacional de Investigaciones Científicas y Técnicas

<sup>3</sup>Instituto Nacional de Tecnología Agropecuaria, Consejo Nacional de Investigaciones Científicas y Técnicas

Bee viruses cause significant losses of honey bee colonies and hence impact on bee products. In previous studies, we detected different viruses in queen rearing apiaries from Argentina. As a promising strategy, RNA interference has been evaluated to control honey bee virus infection. The aim of this study was to evaluate the effect of RNAi technology mediated by double stranded RNA (dsRNA) to reduce Acute bee paralysis virus (ABPV) infection in adult bees.

ABPV gene fragments were used for dsRNA synthesis by in-vitro transcription. To prepare infective virus stock, ABPV was injected in bee pupae and purified by sucrose gradients and ultracentrifugation. Viral presence and abundance were assessed by quantitative real-time PCR. The experiment consisted on the oral administration of dsRNA to adult worker bees, and included five treatments: control (A), specific dsRNA + Virus (B), Virus (C), specific dsRNA (D) and non-specific dsRNA + virus (E). Four replicates per treatment and 50 bees per replicate were performed. The viral quantity used was 1 ml 10<sup>4</sup> genomic copies/replicate, and dsRNA quantity per bee was 1 µg. Bee survival was recorded for 10 days and then, samples were processed for viral quantification. The results indicate that at day 6, there was a significant reduction in the average viral loads in treatment B, compared to treatments C and E. Oral administration of dsRNA reduced the viral replication curve, producing higher bee survival levels in treatment B. On day 5 (2 days post-infection) significant differences were observed in the mean number of live bees between treatment B and treatment E, and from day 6 (3 dpi) between treatment B and treatments C and E. Moreover, treatment B showed no significant differences with controls, regarding bee survival, until day 7. The effect produced by dsRNA administration involved a decrease in average viral loads from 2.48 to 3.48 log cg/µl at time 6 (3 dpi) for each µg of dsRNA administered. This is the first report of ABPV infection reduction in honey bees by oral dsRNA administration. The results of this work support the feasibility of using this methodology to control honey bee virus infection.

### PP-263

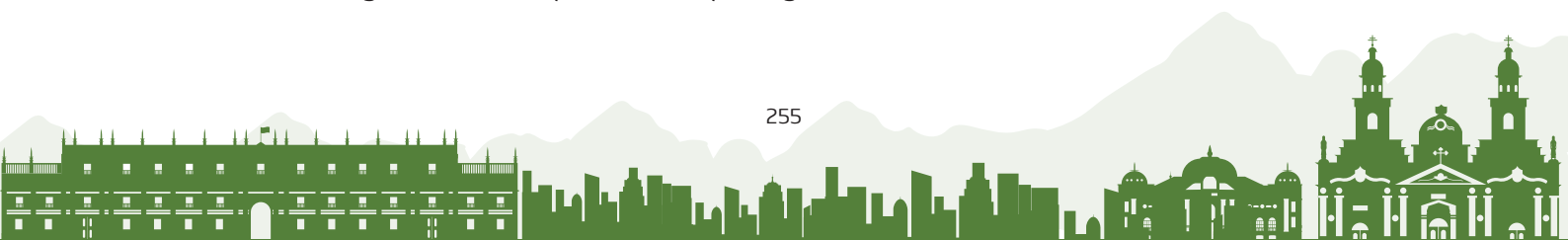
#### Screening for the isolation and characterization of microorganisms with inhibitory capacity of bee pathogens *Apis mellifera* L. Jujuy-Argentina

Gisela Beatriz Ruiz<sup>1</sup>, Rosa Milagro Retamoso<sup>2</sup>, Marcelo Rafael Benítez Ahrendts<sup>1</sup>

<sup>1</sup>Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, INECON, CONICET, San Salvador de Jujuy, Argentina

<sup>2</sup>Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, San Salvador de Jujuy, Argentina

*Apis mellifera* L. is affected by different agents such as viruses, bacteria, fungi and parasites that cause considerable damage in global beekeeping. Some species of ants usually enter *Apis mellifera* hives attracted by the honey and heat generated by the bees, establishing nesting sites between the hive covers. Ants often have microorganisms attached to their cuticles, which can inhibit certain pathogens. The objective of this work was to perform a screening of microorganisms isolated from the cuticle of ants that nest inside *Apis mellifera* hives in order to characterize those with the inhibitory capacity of bee pathogens. Five specimens of eight species of ants were suspended in a 1.5 ml solution of 10% peptone, vortexed for five minutes. 20 µl of the solution were seeded on plates with Nutrient Agar for bacteria at 30°C for 24 to 48 hours and on plates with Potato Glucose Agar medium for fungi at 27°C for seven days. Successive isolations were carried out until obtaining pure strains. Of the microorganisms obtained, those with the ability to inhibit other microorganisms were selected. For this, its characteristics and antimicrobial properties were analyzed. According to this criterion, *Bacillus subtilis* and *Pseudomonas putida* were selected for the inhibition tests. These strains were confronted with the pathogens *Ascosphaera apis*, *Aspergillus section nigri* on MEA and *Paenibacillus* sp. about MYPGP. Both the isolations of the microorganisms and the antagonistic tests were carried out in the Beekeeping and Meliponcola Health Laboratory of the Faculty of Agrarian Sciences of the National University of Jujuy. The inhibition percentages of both bacterial strains against *Ascosphaera apis* and *Aspergillus section nigri* exceeded 50%. The mean diameters of the inhibition halos exceeded 45 mm for *Bacillus subtilis* versus *Paenibacillus* sp. The results obtained show that the selected bacterial strains were the best antagonists, with great potential for the control of numerous fungi and certain *Apis mellifera* pathogens.





### PP-264

## Evaluation Of The Efficacy in the use of Oxalic Acid Against Varroa Destructor in Beehives in the Metropolitan Region Of Chile

Víctor Orlando Castro Cerda<sup>1</sup>, Rafael Alejandro Rodriguez Da Silva<sup>2</sup>, Vania Donoso Solari<sup>1</sup>

<sup>1</sup>Universidad Santo Tomás

<sup>2</sup>Universidad Mayor

In Chile and worldwide, beekeeping has been taking more strength and responsibility every day regarding the care of bees and the use of different products to treat them correctly against various pathologies such as Varroa destructor, which is one of the main problems of productive losses. Regarding the above, a new treatment technique with oxalic acid in papier-mâché slats was proposed, but with a different duration of treatment application. The research was carried out during the months of October 2020 to January 2021, at the Catemito headquarters located in the San Bernardo commune and the laboratory of the Ejército headquarters located in the Santiago commune of the Santo Tomás University, Chile. 12 Langstroth-type beehives were used and divided into 2 groups: Group 1 treatment with oxalic acid on cardboard slats applied every two weeks throughout the productive period and Group 2 original treatment applied at the beginning of the productive season and repeated after two weeks. A Varroa count was performed at the beginning of the treatment (October) and at the end of the months of October, November, December and January. With these results, the % of infestation was calculated, showing that the treatment carried out during the entire productive period is more effective than the original treatment, since the beehives treated only at the beginning ended up with a high % of infestation, which could impair the survival of the hive and the production of these. Finally, the effectiveness of group 1 was determined: end of October 56.98%, end of November 82.33%, end of December 89.57% and end of January, the effectiveness was 42.44%. Efficacy achieved in group 2: end of October 41.09%, end of November 41.15%, end of December and January a null or 0% efficacy was measured, where the parasite loads increased significantly. This confirms that there is a greater effect in the treatment carried out every two weeks during the entire productive period, compared to the traditional treatment or with a single repetition at the beginning of the season.

### PP-265

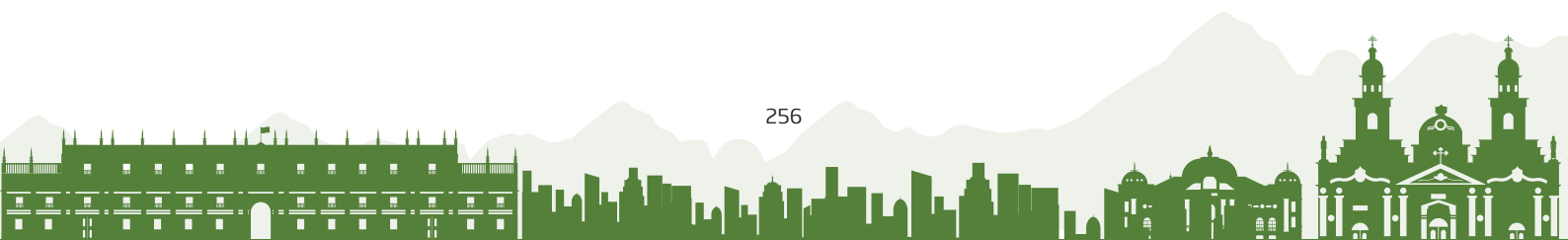
## Probbee: bacterial consortium that strengthens the immune system of honeybees and improves the health and productivity of beehives

Marisol Vargas<sup>1</sup>, Nolberto Arismendi<sup>2</sup>, Macarena Gerding<sup>1</sup>, Diego Silva<sup>1</sup>, Ximena Sepúlveda<sup>1</sup>, Daniel Salamanca<sup>1</sup>

<sup>1</sup>Laboratorios de Virología y Sanidad Apícola, Facultad de Agronomía, Universidad de Concepción, Chillán, Chile.

<sup>2</sup>Centro de Investigación Austral Biotech, Facultad de Ciencias, Universidad Santo Tomás, Valdivia, Chile.

Honey bee colonies have suffered a significant decline in the last decade, with serious consequences for bee production and pollination services. One of the main causes of these losses is related to the presence of pathogens such as the microsporidian *Nosema ceranae* and the deformed wing virus (DWV). The gut microbiota of honeybees, like lactobacteria, has a very relevant role in their health. Therefore, the aim of the study was to develop a stimulant product for honeybees based on bacteria isolated from bee gut, which improves the health and productivity of beehives. Honey bee gut bacteria were isolated from bee colonies distributed in different agroclimatic regions of Chile (North, South, Central regions of the country including Rapa Nui). Thus, 756 strains were isolated, of which 489 were selected, grouped into 17 compatible consortiums, which were tested in vivo in biological assays under controlled conditions. At least 5 consortiums showed to increase survival (39 to 77%), reduce the load of *N. ceranae* (37 to 73%) and DWV titer (37 to 61%) in infected honeybees that were treated with these consortiums and compared to those infected and not treated. Some of these consortiums induced an increase in the relative expression of the antimicrobial peptides abaecin and defensin from 25 to 300% in bees infected with *N. ceranae*, 21 to 67% in bees infected with DWV, and 26 to 71% in mixed infections with both pathogens and compared with those not treated. In field trials, the consortium 9 named as Probbee, which contains 6 strains of lactobacteria (*Lactobacillus kunkeei* and *Bifidobacterium asteroides*), diluted in syrup and applied to the hive feeder, improved the strength of the bee colonies, increasing the bee population up to 160%, compared to those not treated. Also increased the weight of honeybee hive from 38% - 66% in the central southern of Chile (Chillán) to more than 320% in the southern of the country (Río Bueno). The results suggest that Probbee has a positive impact on the control of *N. ceranae* and DWV, stimulates the immune system of honeybees, and improves the strength and productivity of beehives.



### PP-266

#### Study of the inhibitory properties of propolis in bees carrying *Nosema* sp. Jujuy Argentina

Rosa Milagro Retamoso<sup>1</sup>, Gisela Beartiz Ruiz<sup>2</sup>, Lesli Nicole Vallejos Farias<sup>3</sup>, Marcelo Rafael Benitez Ahrendets<sup>2</sup>

<sup>1</sup>Facultad De Ciencias Agrarias Universidad Nacional De Jujuy Argentina

<sup>2</sup>Inecoa-Conicet

<sup>3</sup>Uce Davis Chile

Propolis is a resinous, adhesive substance collected, processed and used by bees from plant species. This product of the hive has numerous biological properties such as antibacterial, antifungal, antiviral, anti-inflammatory, antitumor and antioxidant. Nosemosis is a parasitic disease caused by a microsporidium *Nosema* sp. that develops and affects the digestive tract of adult bees, causing death. The objective of the present work was to determine the inhibitory properties of propolis in bees carrying *Nosema* sp. For this purpose, we worked in two apiaries, Severino and Tilquiza, and the trial was divided into three stages: 1st. Stress conditions were generated in the beehives, through the total closure of the hives for a period of three days. The dilutions of the most effective propolis extract were applied and conveyed by means of a syrup delivered to the bees in the feeders. The treatments were: C (0.3 gr/mL), M1 (0.03 gr/mL) M2 (0.003 gr/mL) and a control T (only syrup). On the fourth day, samples were taken from the bees returning from the flight for subsequent analysis. 2st After the sixth day of stress, bee samples were taken for analysis and the stress conditions in the hives were eliminated. 3st On the ninth day, bee samples were obtained. Five hives were used per trial and the incidence of Nosemosis was evaluated as a function of the concentrations of propolis supplied and the time of the study (initial, average and final). In each of the apiaries, 3 repetitions of the trial were carried out. For both apiaries, depending on the treatments at different times, values from 20,000 to 40,000 spores/bee were obtained in the initial time, and in the mean time there was an increase up to 60,000 spores/bee for all treatments. In the final time, no presence was recorded with treatments C and M1, while the control T maintained the same incidence as in the mean time. The inhibitory capacity of the concentrations of propolis extract on bees carrying *Nosema* sp.

### PP-267

#### Alteration in survival and microbial indicators of "*Apis mellifera*" by constant exposures to fungicide/bactericides in combination with biological stressors

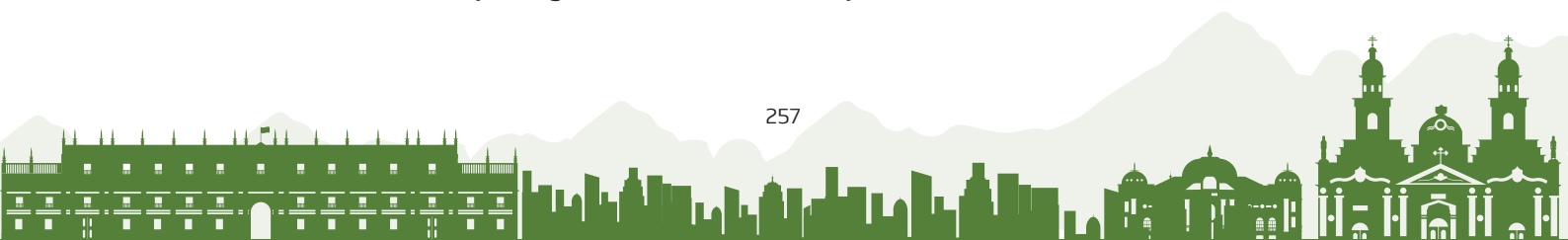
Evelin Troncoso<sup>1</sup>, Miguel Villarroel<sup>2</sup>, Marisol Vargas<sup>3</sup>, Erwin Guzmán<sup>2</sup>, Ernesto Moya<sup>3</sup>, Nolberto Arismendi<sup>2</sup>

<sup>1</sup>Doctorado en Conservación y Gestión de la Biodiversidad, Facultad de Ciencias, Universidad Santo Tomás, Santiago, Chile.

<sup>2</sup>Centro de Investigación Austral Biotech - Laboratorio de Estudios en Abejas, Facultad de Ciencias, Universidad Santo Tomás, Valdivia, Chile.

<sup>3</sup>Laboratorios de Virología y Sanidad Apícola, Facultad de Agronomía, Universidad de Concepción, Chillán, Chile.

To prevent severe losses in fruit production, preventive treatments are carried out between flowering and fruit maturity, including fungicides and bactericides. However, these pesticides could affect honey bees in the process of pollination in fruit trees; an effect that could be aggravated in the presence of common bee pathogens, leading thus, to synergistic effects that cause possible honey bee declines. Thus, the aim of this study was to evaluate the effects on the survival and bacteria associated to gut microbial community of honey bees, exposed to fungicides/bactericides in conjunction with biological stressors such as "*Lotmaria passim*" and deformed wing virus (DWV). Young bees were continuously fed (20 days) with three doses (13, 25 and 624 mg/kg) of copper sulfate pentahydrate (98%) and a mixture of antibiotic streptomycin sulfate (25%) and Oxytetracycline hydrochloride (3.2%) in the absence or presence of biological stressors. The survival rate was higher in bees previously infected with *L. "passim"* (60-70%) compared to those inoculated with DWV (30-55%); in the presence of both pathogens plus the antibiotics the survival rate decreased significantly (20-45%) compared to the control group with only the antibiotic (60%). Copper-derived product showed that, at high doses (624 mg/kg), survival is equal to 0% on the first 10 days of exposure. The range of survival in bees inoculated per separate with pathogens plus copper, showed a similar trend to antibiotics (30-40%), in which case, the survival rate decreased (25-55%) when bees were inoculated with both pathogens at the same time; different from the control group with copper (43-80%). In addition, bacteria associated with the bee gut microbial community are inhibited in their growth by copper-derived products, ranging from 8 to 100% inhibition in "*Apilactobacillus kunkeei*", 21 to 41% in "*Bifidobacterium asteroides*". While antibiotics inhibit the growth of *A. "kunkeei"* (15 to 30%), similar to that observed in *B. "asteroides"* (21 to 32%). This background suggests that copper-derived bactericides/fungicides and antibiotics in conjunction with biological stressors reduce survival, but also affect the bacteria associated with the honey bee gut microbial community.



### PP-268

#### Virome analysis of honey bee colonies after mass disappearance in Korea

Minhyeok Kwon, Chuleui Jung, [Eui-Joon Kil](#)

Department of Plant Medicals, Andong National University, Andong, Republic of Korea

After the nationwide, massive winter losses of honey bees in Korea during the winter of 2021, samplings were conducted from live honey bees in colonies and dead honey bees nearby colonies in the same bee-farms in six regions in Korea. Each sample was subjected to virome analysis using high-throughput sequencing technology. The number of viral reads was the lowest in the live honey bee group sample with 370,503 reads and the highest in the dead honey bee group sample with 42,659,622 reads. Viral contigs were matched with the viral genomes of the black queen cell virus, deformed wing virus, Israeli acute paralysis virus, and sacbrood virus, all of which have been previously reported in Korea. However, Apis rhabdovirus 5, bee macula-like virus, Varroa orthomyxovirus-1, Hubei partiti-like virus 34, Lake Sinai virus 2, 3, and 4, and the Ditton virus, were also discovered in this study, which are the first records in Korea. Plant viral sequences resembling those of Arabidopsis latent virus 1, and a novel viral sequence was also discovered. In the present study 55 complete viral genome sequences were identified. This study is the first virome analysis of domestic honey bees and provides the latest information on the diversity of honey bee viruses in Korea.

### PP-269

#### First metagenomic analysis of virome in Uzbekistan honey bee (*Apis mellifera*): investigating basic information on honey bee viruses

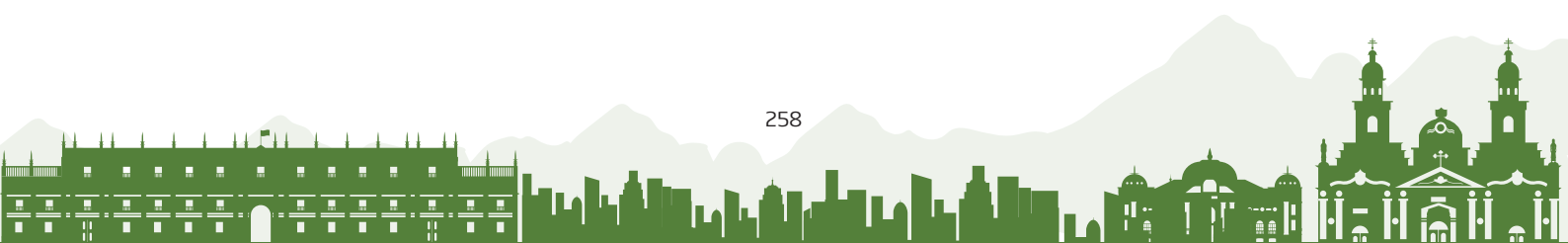
Minhyeok Kwon<sup>1</sup>, Chuleui Jung<sup>1</sup>, Kaxramanov Boymaxmat Abdiazizovich<sup>2</sup>, Rakhimdjanova Nilufar Zakhidovna<sup>3</sup>, [Eui Joon Kil](#)<sup>1</sup>

<sup>1</sup>Department of Plant Medicals, Andong National University, Andong, Republic of Korea

<sup>2</sup>Languages Department, Tashkent State Agrarian University, Tashkent, Uzbekistan

<sup>3</sup>Department of General Zootechnics and Veterinary, Tashkent State Agrarian University, Tashkent, Uzbekistan

Honey bee are economically important insects. However, they face many biotic and abiotic stresses, such as diseases, pesticides, climate change, and pests, which are causing the loss of many honey bee colonies worldwide. Among these factors, viruses have been identified as a major cause of colony loss. In Uzbekistan, research on honey bee viruses has been limited. In this study, an analysis was conducted to investigate the virus affecting honey bees in four cities in Uzbekistan. Virome analysis was conducted from each sample using high-throughput sequencing (Illumina sequencing) and bioinformatics technology. The analysis identified nine honey bee viruses: acute bee paralysis virus, aphid lethal paralysis virus, Apis rhabdovirus 1 and 2, black queen cell virus, deformed wing virus, Lake Sinai virus 4, sacbrood virus, and Hubei partiti-like virus 34. Additionally, 20 plant viruses were identified, nine of which were novel. This experiment is the first virome analysis using Uzbekistan honey bees and provides a foundation for understanding the viruses affecting honey bees in Uzbekistan.



## Bee Health

### PP-270 The INSIGNIA-EU project

**Flemming Vejsnaes**<sup>1</sup>, Norman L. Carreck<sup>2</sup>, Konstantinos M. Kasiotis<sup>4</sup>, Ivo Roessink<sup>5</sup>, Robert Brodschneider<sup>6</sup>, Valters Brusbardis<sup>7</sup>, M. Alice Pinto<sup>3</sup>, Amadeo R Fernández Alba<sup>9</sup>, Dirk C. De Graf<sup>11</sup>, Kristina Gratzner<sup>6</sup>, Alison Gray<sup>12</sup>, Fani Hatjina<sup>8</sup>, Andreia Quaresma<sup>3</sup>, Maria Murcia Morales<sup>9</sup>, Marco Pietropaoli<sup>10</sup>, Ole Kilpinen<sup>1</sup>, Bas Buddendorf<sup>5</sup>, Ellen Danneels<sup>11</sup>, Sjef Van Der Steen<sup>13</sup>

<sup>1</sup>Danish Beekeepers Association, Sorø, Denmark

<sup>2</sup>Carreck Consultancy Ltd, Shipley, UK

<sup>3</sup>Centro de Investigação de Montanha Instituto Politecnico de Bragança, Bragança, Portugal

<sup>4</sup>Benaki Phytopathological Institute, Athens, Greece

<sup>5</sup>Stichting Wageningen Research Wageningen, Netherlands

<sup>6</sup>University of Graz, Graz, Austria

<sup>7</sup>Latvian Beekeepers Association, Jelgava, Latvia

<sup>8</sup>Ellinikos Georgikos Organismos - Dimitra, Nea Moudania, Greece

<sup>9</sup>Universidad De Almeria, Almeria, Spain

<sup>10</sup>Instituto Zooprofilattico Sperimentale Delle Regioni Lazio E Toscana, Rome, Italy.

<sup>11</sup>University of Gent, Gent, Belgium

<sup>12</sup>University of Strathclyde, Glasgow, UK

<sup>13</sup>Alveus AB Consultancy, Oisterwijk, Netherlands

The INSIGNIA-EU study is a pan-European study, conducted in all 27 EU countries. It describes the pollution status of the environment in the proximity of an apiary. It uses the honey bee colony as an environmental monitoring tool, because the pollutants found on bees and bee products reflect the pollution status around the apiary. The study can detect pesticides, microplastics, heavy metals, and air pollutants. We are sampling the colonies non-invasively, using in-hive-passive samplers. In the 27 EU countries, beekeeper citizen scientists in 320 apiaries are sampling bi-weekly at the same time and in the same way from May till the end of August 2023. This environmental monitoring provides comparable spatial and temporal data. Bi-weekly pollen is also collected and analyzed molecularly for its botanical origin. The pollution- and pollen data will then be statistically analyzed and INSIGNIA spatial and temporal pesticide risk exposure- and pollen availability models will be built.

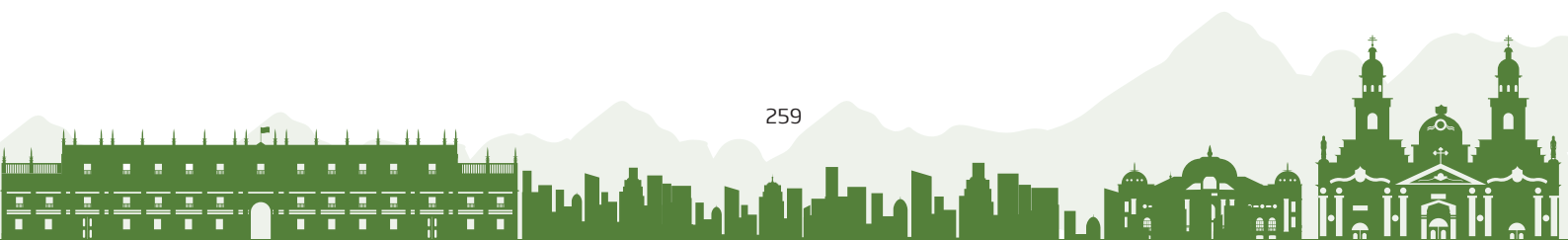
### PP-271 Phenotypic and genotypic resistance to colistin in environmental bacteria isolated in Emilia-Romagna by biomonitoring with *Apis mellifera* colonies

**Giovanni Cilia**<sup>1</sup>, Ilaria Resci<sup>2</sup>, Laura Zavatta<sup>1</sup>, Silvia Piva<sup>2</sup>, Elisabetta Mondo<sup>2</sup>, Sergio Albertazzi<sup>1</sup>, Valeria Caringi<sup>1</sup>, Antonio Nanetti<sup>1</sup>, Laura Bortolotti<sup>1</sup>

<sup>1</sup>CREA Research Centre for Agriculture and Environment, Bologna, Italy

<sup>2</sup>Department of Veterinary Medical Sciences, Alma Mater Studiorum, Bologna University, Ozzano Emilia (BO), Italy

Colistin is an antimicrobial polymyxin mainly used in clinical settings against infections by multi-resistant Gram-negative bacteria. The nine genes coding for colistin resistance mechanisms (*mcr*), generally are transmitted horizontally via mobile plasmids. To date, the spread of resistant bacteria and the presence of these genes in the environment is poorly investigated. The behavioural and morphological peculiarities of *Apis mellifera* have already favoured the use of their colonies as environmental bioindicators of both resistance genes and antibiotic-resistant bacteria. The study aimed to evaluate the phenotypic and genotypic resistance to colistin of Gram-negative bacteria spread in the environment and, isolated from the body surface and gastrointestinal tract of foraging bees. Bees were sampled from a network of 33 colonies sited throughout the Emilia-Romagna region as part of the BeeNet project. Phenotypic resistances of bacteria were determined through a microdilution assay by evaluating the minimum inhibitory concentration (MIC) through dilutions of the antimicrobial from 0.5 µg/ml to 256 µg/ml. Strains with MIC values > 2 µg/ml were considered resistant. The presence of the nine *mcr* genes was performed by two different multiplex PCRs (one for *mcr1*-*mcr5* genes, the other for *mcr6*-*mcr9*). Among isolated strains, 68% were resistant to colistin, and of these, 21% had MIC values > 256 µg/ml. The *mcr2* and *mcr5* genes were the most frequent, in 14% and 28% of the strains, respectively. With a subsequent land-use analysis, it was also possible to create a predictive model to correlate antimicrobial resistance with land-use characteristics. Wetlands, livestock, and crops showed a higher probability of isolation of colistin-resistant bacteria. The results obtained indicate the possibility of both identifying the presence of certain colistin resistance genes in bacteria spread throughout the environment and their distribution at the environmental level, although their use is strictly limited in clinical settings. This environmental monitoring takes great utility, especially in the One-Health approach, given the sanitary importance of colistin.



### PP-272

#### Population dynamics of the mite *Varroa destructor* in africanized bees in the brazilian semiarid

Katia Peres Gramacho<sup>1</sup>, Leandro Alves Da Silva<sup>1</sup>, Herica Tertulino<sup>1</sup>, Lucas Da Silva Morais<sup>1</sup>, Anderson Dias Da Silva<sup>1</sup>

<sup>1</sup>Leandro Alves da Silva

<sup>2</sup>Anderson Dias da Silva

<sup>3</sup>Hérica Girlane Tertulino Domingos

<sup>4</sup>Lucas da Silva Morais

<sup>5</sup>Kátia Peres Gramacho

Varroaosis is a disease present in Africanized bees in all states of Brazil. This disease, caused by the ectoparasite *V. destructor*, is considered the most lethal pest in the world; however, in Brazil, it did not cause significant damage to beekeeping, as the Africanized bees quickly developed tolerance to this parasite, maintaining an infestation rate of approximately 3% and ruling out the need for chemical treatment for population control. However, the infestation rate varies according to season, with its peak being reached during periods of food scarcity. Most of the published data that show the infestation rate, mainly in the Northeast of Brazil, were collected in just three or four months in the off-season. Considering the lack of broader data on mite infestation rates in Africanized bees, the present study aimed to evaluate the *V. destructor* infestation rate between January and December 2020 in hygienic and non-hygienic colonies in the semi-arid region of Brazil. For this, the brood drilling method was applied to 37 colonies, and two groups were formed: G1- hygienic colonies with 16 colonies and G2- unhygienic colonies with 21 colonies. After the groups were formed, approximately 300 adult bees were collected monthly from each colony to analyze the infestation rate. Monthly climate data were also collected [temperature (T °C), relative humidity (RH%), and rainfall (PPmm)]. The average monthly infestation can be considered low, considering that it remained below 10%, with the exception of November, which reached 12.19%±6.45%; however, there was variation in infestation between colonies, reaching 42%. There was no statistical difference in the infestation rates between the phenotypic groups (P>0.05). It was observed that the mite infestation associated with the climatic variables influenced the swarming of 32% of the colonies (T°C= 0.3522; UR= -0.40398; PPmm= -0.3981). There was no significant correlation between hygienic behavior and monthly infestation of this parasite, showing that the tolerance of Africanized bees is the result of several factors and not just hygienic behavior. Even so, the results reinforce the line of thought that, in Brazil, there is no need to apply acaricides to control *V. destructor*.

### PP-273

#### Is DWV-B replacing DWV-A in Poland?

Anna Gajda<sup>1</sup>, Ewa Mazur<sup>1</sup>, Hajnalka Szentgyörgyi<sup>2</sup>, Aleksandra Żmuda<sup>2</sup>, Matthias Albrecht<sup>3</sup>, Peter Neumann<sup>4</sup>, Robert Paxton<sup>5</sup>, Oliver Schweiger<sup>6</sup>, Josef Settele<sup>7</sup>, Adam Vanbergen<sup>8</sup>

<sup>1</sup>Laboratory of Bee Diseases, Institute of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Warsaw, Poland

<sup>2</sup>Institute of Botany, Jagiellonian University, Kraków, Poland

<sup>3</sup>Agroecology and Environment, Agroscope, Zürich, Switzerland

<sup>4</sup>Institute of Bee Health, University of Bern, Bern, Switzerland

<sup>5</sup>Institute of Biology, Martin Luther University Halle-Wittenberg, Germany

<sup>6</sup>UFZ Helmholtz Center for Environmental Research, Halle (Saale), Germany

<sup>7</sup>UFZ Helmholtz Center for Environmental Research, Leipzig, Germany

<sup>8</sup>INRAE-National Research Institute for Agriculture, Food and Environment, France

Insect pollinators face multiple threats, with diseases a potentially leading cause of mortality. One of the most common disease risks comes from Deformed Wing Virus type A and B. In most European countries type B is much more prevalent than type A, at least in *Apis mellifera*, wild bees remain underresearched still. In Poland the data in the prevalence of each type in honeybees is very scarce. In wild bees non-existent.

In this study, we examined honeybees and wild bees (of different species) collected during spring, early summer and late summer of 2021, directly from flowers from transects in the Lesser Poland region. We checked for the presence of DWV-A and DWV-B, and for infection intensity with common molecular biology methods.

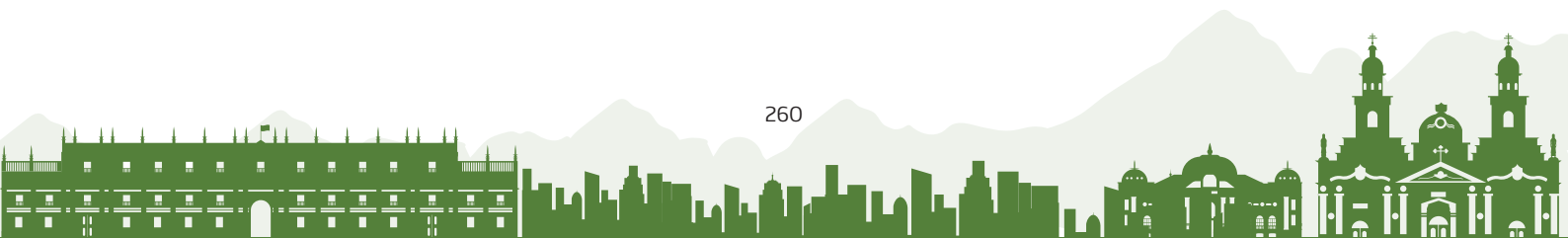
Results showed, that during the spring of 2021 100% of honeybees, and 100% of wild bees were infected with DWV-A with the infection intensity higher in honeybees (mean viral titres: 5080864 and 1575). DWV-B was found in 55% of honeybees (mean viral titres: 667) and zero wild bees.

Early summer brought a drop in DWV-A infected bees. For honeybees it was 75%, and for wild bees - 52% with the respective mean viral titres of 128236 and 30707. DWV-B was however not found in any *Apis mellifera* and in 45% of wild bees (mean titre: 173).

Late summer showed an even more significant drop in DWV-A infected insects. For *Apis mellifera* it was 6%, and wild bees - 3% (with also a very low mean titres: 51 and 107). DWV-B was found in 95% of honeybees and 89% wild bees (with mean titres respectively 4325 and 64914).

The numbers suggest, that DWV-A is being replaced by DWV-B in wild and managed bees during the season. In early summer the wild bees seem to „catch“ it first, which is very surprising, given that usually viruses are transmitted from honeybees to wild bees. In late summer DWV-A almost disappears and DWV-B becomes the most prevalent one, however, the titres are not high.

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### PP-274

#### Effects of individual and combined exposure to sublethal doses to the pesticides Chlorpyrifos, Bifenthrin and Imidacloprid on detoxification and neurotoxicity enzymes in *Apis mellifera* workers

Tamara Perezlindo<sup>1</sup>, Azucena Iglesias<sup>1</sup>, Gaston Iturburu<sup>2</sup>, Silvina Quintana<sup>1</sup>, Matias Maggi<sup>1</sup>, Edgardo Sarlo<sup>1</sup>, Mirta Menone<sup>2</sup>, Martin Eguaras<sup>1</sup>, Sandra Medici<sup>1</sup>

<sup>1</sup>. Centro de investigación en abejas sociales (CIAS), Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), Consejo Nacional e Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Mar del Plata UNMDP). Centro de asociación simple CIC PBA. Mar del Plata, Buenos Aires, Argentina

<sup>2</sup>. Instituto de Investigaciones Marinas y Costeras (IIMYC) CONICET, UNMDP, Argentina.

The honeybee (*Apis mellifera* L.) is an important pollinator and a model for pesticide effects on insect pollinators. Bees can be exposed to multiple pesticides that may interact synergistically, amplifying their side effects. Some combinations show synergistic or antagonistic combined effects that go far beyond what is predicted with current effect models. Up until now, only the combined additive effects of similar acting chemicals have been assessed accurately, whereas the combined effects of dissimilar acting chemicals have been greatly underestimated in many cases. In recent years, the mortality of bees, such as *Apis mellifera*, has become a strange global phenomenon that is having a negative impact given its great importance. The aim of this work was to analyze the impact of exposure to different agrochemicals for agricultural use in sublethal doses and individually and in combination, on detoxification enzymes (Catalase (CAT) and Glutathione-S-transferase (GST)) and neurotoxicity (Acetylcholinesterase (AChE)). Bees up to 3 days old were collected and divided to carry out the different treatments, using 10 bees in each replicate. 10 µl of Bifenthrin (BIF), Chlorpyrifos (CLOR) and Imidacloprid (IMI) were applied topically in concentrations corresponding to LD10 (The range of concentrations used was based on previous studies) as follows: 1) BIF; 2) CLOR; 3) IMI; 4) BIF+CLOR; 5) BIF+IMI; 6) IMI+CLOR; 7) CONTROL. After each treatment, the surviving bees were kept in a freezer at -80°C for their subsequent enzymatic analysis. The results obtained showed that for GST there is a significant inhibition for all treatments with the control ( $p < 0.05$ ), except for the IMI+CLOR combination where there is no significant difference ( $p > 0.05$ ). For the CAT enzyme all treatments were significantly lower than the control ( $p < 0.05$ ). In the case of AChE, the individual treatments of IMI, BIF, and CLOR as well as the IMI+CLOR combination were lower than the control ( $p < 0.05$ ) while the BIF+CLOR combination was not different from the control ( $p < 0.05$ ) and the IMI+BIF combination was significantly superior. These results report the synergistic (IMI+BIF for the AChE enzyme) and antagonistic (IMI+CLOR for GST and AChE) effects caused by simultaneous exposures to pesticide combinations in bees.

### PP-275

#### Towards sustainable and productive apiculture: Selection of resilient honeybees free of zoosanitary products (ECOAPI)

Melanie Parejo<sup>1</sup>, Luis Javier Chueca<sup>1</sup>, Egoitz Galartza<sup>2</sup>, Xose Manuel Durans<sup>3</sup>, June Gorrochategui-Ortega<sup>1</sup>, Kaira Wagoner<sup>4</sup>, Andone Estonba<sup>1</sup>, Iratxe Zarraonaindia<sup>5</sup>

<sup>1</sup>Applied Genomics and Bioinformatics, University of the Basque Country (UPV/EHU), Leioa, Spain

<sup>2</sup>ERBEL, Iberian Bee (Erle Beltz) Breeding Association, Zaldibia, Spain

<sup>3</sup>MENA beekeeping association, Santiago de Compostela, Spain

<sup>4</sup>Department of Biology, University of North Carolina Greensboro, Greensboro, NC, USA

<sup>5</sup>IKERBASQUE Basque Foundation for Science, Bilbao, Spain

The beekeeping sector in Spain is the largest in Europe but suffers from large annual honeybee colony losses driven by climate change and pests and parasites, with *Varroa destructor* and associated viruses being a major concern. Varroosis requires recurring chemical treatments and thus the sector is in need of finding more eco-friendly solutions.

Within the ECOAPI project (2022 - 2024), we aim at a long-term sustainable solution against varroosis based on the selection of varroa-resistant lines that could avoid or minimize chemical treatments. Varroa resistance can be achieved by natural, or targeted selection. Within ECOAPI, we study two populations of *Apis mellifera iberiensis*: a putative varroa-resistant population ("MENA population"), that consists of colonies being untreated since 2015 and managed by the beekeeping association MENA; and a breeding population ("ERBEL population") managed by the bee breeding association ERBEL within a genetic improvement program that selects for varroa resistance among other performance traits. In the untreated "MENA population", we study the factors (environment, genome, microbiome) and mechanisms (social immunity, management) that enable Varroa resistance and survival. In the "ERBEL population", we apply and evaluate a new pheromone-based test (UBeeO) for hygienic behaviour specifically against varroa-parasitized brood that promises to be a faster and more efficient measurement to predict colony-level Varroa resistance than commonly used strategies, and it could therefore be implemented in breeding programs. We will correlate it with Varroa infestation levels across the season, social immunity traits and other colony performance parameters collected within the breeding program. Moreover, we aim to identify genetic markers associated with varroa-resistance that could lead to genetic tools employed in breeding of resistant stocks across the country.

In conclusion, the ECOAPI study will evaluate for the first time the traits involved in varroa resistance of Iberian honeybees. The results will provide know-how on the major factors and mechanisms of colony losses through varroosis, enabling adapted low-input management or breeding solutions that will lead the beekeeping sector towards sustainable production without the use of synthetic acaricides.



### PP-276

#### Agricultural management intensity has an imprint on the Apibiome: implications for bee health

June Gorrochategui-Ortega<sup>1</sup>, Marta Muñoz Colmenero<sup>2</sup>, Marin Kovačić<sup>3</sup>, Janja Filipi<sup>4</sup>, Zlatko Puškadija<sup>3</sup>, Nikola Kezić<sup>5</sup>, Melanie Parejo<sup>1</sup>, Ralph Böhler<sup>6</sup>, Andone Estonba<sup>1</sup>, Iratxe Zarraindia<sup>7</sup>

<sup>1</sup>Department of Genetics, Physical Anthropology and Animal Physiology, Applied Genomics and Bioinformatics, University of the Basque Country (UPV/EHU), Leioa, Spain

<sup>2</sup>Department of Genetics, Physiology and Microbiology, Complutense University of Madrid, Madrid, Spain

<sup>3</sup>Faculty of Agrobiotechnical Sciences, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

<sup>4</sup>Department of Ecology, Agronomy and Aquaculture, University of Zadar, Zadar, Croatia

<sup>5</sup>Department of Fisheries, Apiculture and Special Zoology, University of Zagreb, Zagreb, Croatia

<sup>6</sup>Landesbetrieb Landwirtschaft Hessen, Kirchhain, Germany

<sup>7</sup>IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

The apibiome, the microbial communities present within hive compartments and bees, shapes honey bee health. External factors (e.g. agricultural intensity and pesticides, low floral composition) can adversely affect bee health after inducing a microbial imbalance, named dysbiosis. However, it is unclear how this dysbiosis develops at the apibiome level, or how fast microbial balance is restored. To deepen our knowledge in those topics, we have monitored 43 hives located across an anthropization gradient (agricultural, semi-natural and natural site) for 3 years. In addition, throughout the trial some of the hives were transferred from the agricultural to first the semi-natural area and later to the natural location. The results from our first year sampling showed that the apibiomes at the agricultural site suffered a depletion of beneficial bacteria. A fast alleviation of the microbial dysbiosis was observed after transferring hives from the agricultural to the semi-natural landscape. This rapid microbial adaptability evidenced in the study is a stepping stone in the development of restorative or palliative methods to strengthen bee health. Furthermore, the long-term microbial monitoring conducted across seasons will enable the detection of global patterns of anthropization and of core microbes of the apibiome. It will also contribute towards the identification of (1) beneficial profiles that could be targeted to strengthen honey bee health at any time-point, (2) bacteria that weaken colonies, and (3) biomarkers that indicate the risk status of hives under anthropization.

### PP-277

#### Modulation of gut microbiota in worker honey bees experimentally infected with *Nosema ceranae*

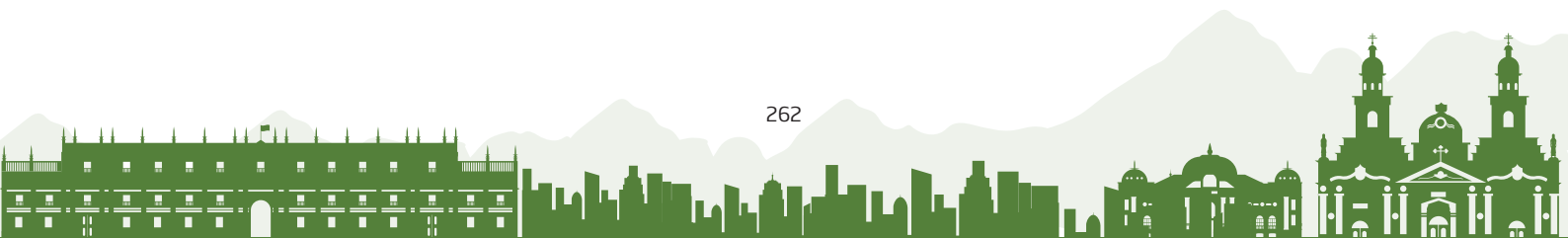
Raquel Martín Hernández<sup>1</sup>, Daniel Aguado López<sup>1</sup>, Almudena Urbieto Magro<sup>1</sup>, Mariano Higes Pascual<sup>1</sup>, Juan Miguel Rodríguez Gómez<sup>2</sup>

<sup>1</sup>IRIAF—Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal, Centro de Investigación Apícola y Agroambiental (CIAPA), Marchamalo, Spain

<sup>2</sup>Departamento de Nutrición y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid (UCM), Madrid (Spain)

*Nosema ceranae* is an obligate intracellular parasite that infects the midgut cells of honey bees. Previous studies showed that younger adult worker bees were more susceptible to infection than older ones. In this paper we analyse how the age of infection with the parasite modifies the establishment of gut bacterial communities. For this purpose, qRT-PCR was used to analyse the main bacteria that form part of the normal intestinal microbiota in adult worker bees of different ages. These bees were infected in the laboratory with the same number of *N. ceranae* spores and sacrificed after 7 days. The results were compared with uninfected bees as a control group. The *N. ceranae* spore solution used to infect the bees was also tested for the presence of bee gut bacteria. A preliminary analysis of the results showed that *Gilliamella apicola*, *Lactobacillus Firm-4* and *Firm-5*, *Bifidobacterium asteroides* and *Bartonella apis* were part of the gut microbiota of all bees (both infected and controls) of all ages. However, *Snodgrassella alvi*, *Frischella perrara* and *Bombella apis* were not detected at some ages. There was a higher number of bees positive for all bacteria when bees were infected just after emergence. Spore solutions were negative for all bacteria. In terms of bacterial load, the highest level of *G. apicola* was found in bees infected just after emergence (both infected and uninfected). *S. alvi* and *Lactobacillus Firm-5* showed higher loads when bees were infected on days 11 and 14 a.e. The load of *B. asteroides* was highest in older infected bees and that of *B. apis* in older controls. The *Lactobacillus Firm-4* load was highest in bees infected at 1 day a.e. and *F. perrara* was highest in bees infected at day 4 and 14 a.e. *Bo. apis* recorded its highest load in bees infected at day 13 a.e. These results suggest that experimental infection with *N. ceranae* may influence and modulate the establishment of the honey bee gut microbiota.

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### PP-278

#### Effects of field realistic doses of pesticides on honey bees

Loreley Castelli<sup>1</sup>, Ciro Invernizzi<sup>2</sup>, Sheena Marie Salvarrey<sup>2</sup>, Horacio Heinzen<sup>3</sup>, Franco Teixeira de Mello<sup>4</sup>, Silvina Niell<sup>5</sup>, Karina Antúnez<sup>1</sup>

<sup>1</sup>Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, IIBCE.

<sup>2</sup>Sección Etología, Facultad de Ciencias. Universidad de la República. Montevideo, Uruguay.

<sup>3</sup>Farmacognosia y Productos Naturales, Facultad de Química. Universidad de la República. Montevideo, Uruguay

<sup>4</sup>Departamento de Ecología y Gestión Ambiental. Centro Universitario Regional Este. Universidad de la República. Maldonado, Uruguay.

<sup>5</sup>CENUR Litoral Norte. Universidad de la República. Paysandú, Uruguay.

Honey bees play an essential role in the environment, being important pollinators contributing to agricultural production and maintaining natural ecosystems. However, large-scale honey bee colony losses and drops in productivity have been reported worldwide, and pesticides exposure is one of the potential causes. Monitoring campaigns in Uruguay showed the simultaneous occurrence of multiple pesticide residues at sublethal levels, the effect of this mixtures on bee physiology has been scarcely studied. In this study we evaluated the impact of chronic exposure to realistic sublethal doses of pesticides on bee physiology and survival. In particular we focused on the herbicide Metolachlor, the fungicide Azoxystrobin, the insecticide Chlorantraniliprole and their equimolar mixture. Two doses were evaluated, D1(0.005, 0.1 and 0.006 mgkg<sup>-1</sup> respectively), the doses detected in pollen in Uruguay in previous studies, and D2, ten times higher. We caged newly emerged worker honey bees in groups of 30, and fed them with contaminated sugar syrup ad libitum. A positive control was included with bees exposed to Dimethoate (insecticide), as a negative control (non-contaminated syrup). The experiment was carried out by triplicate. Besides that, a second experiment was conducted adding irradiated pollen to all groups. Sugar syrup consumption and survival were recorded daily. At day 10, 5 bees/cage were sampled to evaluate dry weight of the head, as a marker of hypopharyngeal gland size, and gut microbiota. In both experiments, exposure of honey bees to different doses of pesticides and their mixtures did not alter the risk of death or survival. However, in all cases we observed a significant reduction in the dry weight of their heads compared to the control group. Those results suggest that pesticide exposure negatively affects the size of hypopharyngeal glands, probably affecting the production of royal jelly, food for the larvae and the queen. Future studies will focus on the impact on gut microbiota. Those results may help to understand sublethal negative impacts of pesticides on bees on agricultural environments.

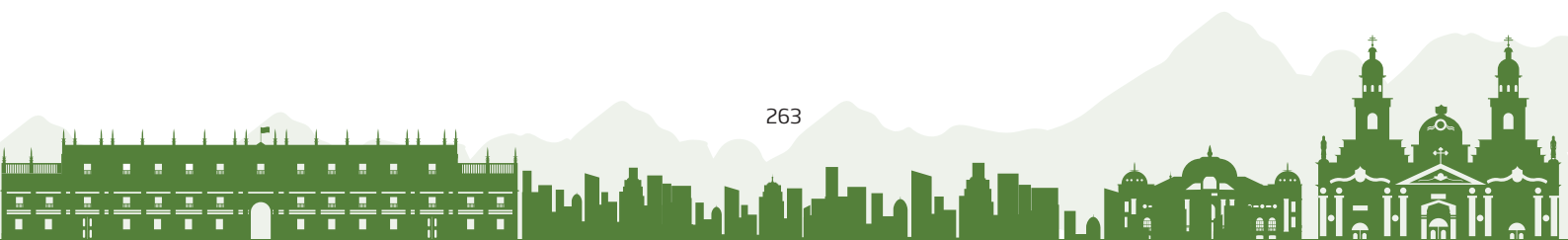
### PP-279

#### Factors affecting honey bee colonies (*Apis mellifera* Linnaeus, 1758) in agricultural areas: presence of pathogens and food's nutritional quality

Amelia V. González-Porto, Daniel Aguado López, Soledad Sagastume, Nauzet Dorta García, Mariano Higes, Raquel Martín Hernández

Centro de Investigación Apícola y Agroambiental (CIAPA) de Marchamalo - Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRAF)

Honey bees (*Apis mellifera*, Apoidea: Anthophila) are one of the main pollinators of flowering plants and as such play a key role in ecosystem dynamics. Colonies around the world are experiencing losses and several factors have been proposed as responsible, including the presence of pathogens such as the Varroa destructor mite, the microsporidium Nosema ceranae or viral infections such as those caused by the Deformed Wing Virus (DWV), the Acute Bee Paralysis Virus (ABPV) or Black Queen cell Virus (BQCV). The incidence, seasonality and virulence of these pathogens may be the result of their own interaction or of synergistic effects in relation to the type and quality of the hive diet. In this work, 35 hives in the province of Guadalajara (Spain) were studied during one year, determining the prevalence of pathogens and the quality of the diet. This study confirms the seasonality already described for *N. ceranae*, the regular presence of virus in hives and the correlation of this microsporidium with DWV. The influence of colony nutrition are discussed to determine whether nutrition influences pathogen levels. A better understanding and study of the interactions between the different factors affecting colony loss may help to develop plans to improve the survival of bee populations worldwide.



## Bee Health

### PP-280

#### Bee samples from Swiss and American museums contain the *Apis mellifera* filamentous virus (AmFV)

**Andrés Delgado-Cañedo, Gustavo Carvalho Nascimento**

Programa de pesquisa e extensão em apicultura do Pampa (APIPAMPA), Universidade Federal do Pampa - Campus São Gabriel, São Gabriel, RS, Brasil.

*Apis mellifera* filamentous virus (AmFV) is a virus that has not yet been classified in a viral family. It was the first bee virus to be found. It has a big DNA genome and is linked to lechoso hemolymph disease. Additionally, there are only three full sequences of this virus currently available. Getting new complete genomes would help us learn more about its variants, how they affect the infected cells, and how they evolve over time. In this work, we examined high-throughput sequencing data of bee samples from Swiss and American museums obtained from SRA database (NCBI) [ERR5665177 (1941), ERR5665174 (1947), ERR5665172 (1954), ERR5665181(1959)] and [SRR5580778(2002), SRR5580780(1999), SRR5580782(2002), SRR5580783(1999), SRR5580787(1999), SRR5580794(1910), SRR5580801(1983), SRR5580805(2014), SRR5580819(1966), SRR5580827(1966), SRR5580831(2014), SRR5580834(2010), SRR5580850(1968), SRR5580854(1966), SRR5580855(1966), SRR5580857(1966), SRR5580859(1966), SRR5580862(2014)], respectively. The mapping of the reads, genome assembly, and alignments were made with the Galaxy server's BOWTIE2, Pylon, and MAFFT tools (usegalaxy.org). In all of the tests, the KR819915 sequence was used as the virus's reference genome. The VCF output files from the Pylon tool were used to determine the coverage. The results have shown variable regions. One of these regions is the region between 485Kpb and 490Kpb, which is very repetitive and thymine-rich. The viral genome sequence has changed over time, as shown by the phylogenetic study of these samples. The SNPs and INDELS that make each variant unique show that the virus has changed between the oldest sample from 1910, which is the most different from the reference genome in terms of evolution, and the most recent sample from this experiment (2014). The information that has been collected so far shows that it is possible to get complete genomic sequences of the AmFV viruses in museum samples and study how their molecular structures have evolved over time. At the same time, the viral sequence of the SRR5580794 sample, from 1910, can be used to date the oldest sequence of AmFV.

### PP-281

#### Determination of neonicotinoid residues in comb honey and beeswax from Croatia using high performance liquid chromatography - tandem mass spectrometry

**Damir Pavliček<sup>1</sup>, Ivana Tlak Gajger<sup>2</sup>, Nina Bilandžić<sup>3</sup>, Maja Đokić<sup>3</sup>, Ivana Varenina<sup>3</sup>, Marija Sedak<sup>3</sup>**

<sup>1</sup>Croatian Veterinary Institute, Veterinary Institute Krizevci, Krizevci, Croatia

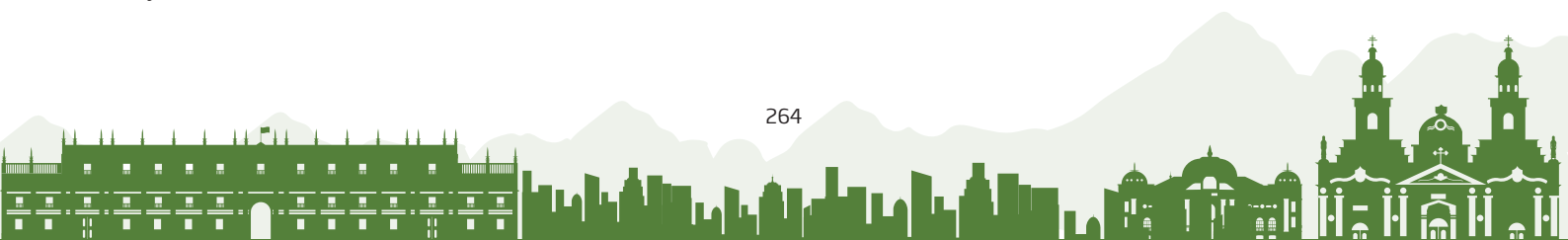
<sup>2</sup>Faculty of Veterinary Medicine Zagreb, Zagreb, Croatia

<sup>3</sup>Croatian Veterinary Institute Zagreb, Zagreb, Croatia

Since their introduction into the market in the 1990s, neonicotinoid pesticides have been widely applied for controlling pests in a variety of agricultural crops. The extensive or inappropriate use by farmers and slow degradation of these compounds in the environment can lead to the contamination of the ecosystem in which the honeybees operate. Therefore, the distribution of neonicotinoids in the environment needs to be adequately assessed in order to better assess the risks for bees and other insect pollinators. Also, contaminants can be transported by usual forager activity to the hive, where they can be transferred into honey. Not only that the presence of such xenobiotics in honey and other honeybee products can decrease their quality and devalue their properties, but it also represents a possible human exposure route to these systemic pesticides.

In this study, comb honey samples taken from hives in geographically dispersed apiaries in Croatia were analyzed for the presence of neonicotinoid residues. Since the honey had to be extracted from the comb prior to analysis, the determination of the target analytes in the residual raw wax samples was also performed. A multi-residue method included QuEChERS-based sample preparation followed by reverse-phase liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for simultaneous detection and quantification of 7 neonicotinoids and 4 imidacloprid metabolites in the observed honeybee matrices. The method was fully validated in terms of selectivity, linearity, precision and recovery, fulfilling the requirements of the SANTE document. Low limits of quantification (LOQ) could be achieved for analytes ranging 0.2-1.0 ng/g in comb honey, and 0.2-2.0 ng/g in beeswax, respectively.

In total, 49 samples of comb honey and beeswax collected in 2020 were analyzed. Among them 8 samples of comb honey and 11 samples of beeswax contained at least one neonicotinoid residue above LOQ. Thiacloprid was the most frequently detected compound. Since a multitude of studies have demonstrated the detrimental effects of neonicotinoids on bees' health, levels of thiacloprid residues obtained in comb honey (107.7 ng/g) and beeswax (165.3 ng/g) from the Međimurje county could be associated with a great increase in the colonies mortality in that region, recently.





### PP-282

## Varroa destructor infestation in pre-pupae and pupae of Apis mellifera in Pantanal, Brazil, 2018-2021

Vanderlei Doniseti Acastio Dos Reis<sup>1</sup>, Vanderlei Doniseti Acastio Dos Reis<sup>2</sup>, Carlos Adriano Ojeda Salles<sup>3</sup>, Simone França Lemes Fernandes<sup>3</sup>

<sup>1</sup>Embrapa Pantanal (Brazilian Agricultural Research Corporation/Pantanal)

<sup>2</sup>Nuclear Energy Center for Agriculture, University of São Paulo (CENA/USP)

<sup>3</sup>Biological Sciences

The *Varroa destructor* mite is an ectoparasite that feeds by sucking the hemolymph from pupae, pre-pupae and adults of Africanized bees (*Apis mellifera*). The process of parasitism exerted by *V. destructor* results in various damages to affected individuals, ranging from malformation, reduced longevity, possible transmission of viruses and, in cases of greater infestation, death. With this study, we sought to determine the infestation rate in capped broods (pre-pupae and pupae) during the seasons of the year in the two castes of adult bees: workers and drones in five colonies in an apiary of Embrapa Pantanal located in the Nhumirim ranch (18°59'15.70"S, 56°37'09.30"W), sub-region of Nhecolândia of Pantanal, in Corumbá-MS, Brazil. The collections were from October 2018 to March 2021. The quantification of the level of parasitism was based on the removal of parts of capped broods from one or two combs of each colony with approximately 100 cells. The formula used to obtain the infestation rate in capped broods (pupae and pre-pupae) was: Infestation rate (%) = (Number of mites/Number of cells) x 100. The months were divided according to the seasons of the year, spring: October, November and December; summer: January, February and March; autumn: April, May and June, and winter: July, August and September, it should be noted that the first day of each month was adopted as the beginning of these seasons. The acquired results were expressed as mean (%) and standard deviation. In the few months in which drone pupae and pre-pupae were collected, the highest infestation was obtained in spring (October) 2019 with an average of  $27.83 \pm 32.39$  and the lowest in autumn (June) 2019 with average of  $0.16 \pm 1.07$ . The results for worker chicks indicated a higher infestation rate in autumn (May) 2020 with an average of  $10.71 \pm 4.56$  and the lowest in winter (August) 2020 with an average of  $0.11 \pm 0.24$ . The results determined that the level of infestation in pre-pupae and pupae throughout the year is greatly influenced by the presence or absence of drone broods.

### PP-283

## Effect of the exposure with sublethal dosis to the insecticides Imidacloprid, Bifenthrin and Chlorpyrifos or its combination on the total amount of body proteins and mRNA expression levels of Vitelogenin on Apis mellifera workers bees

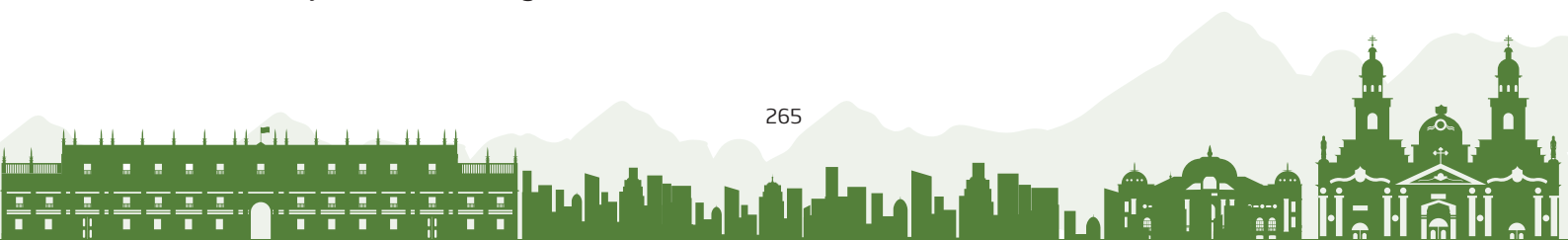
Edgardo Sarlo<sup>1</sup>, Silvina Quintana<sup>1</sup>, Tamara Perezlindo<sup>2</sup>, Azucena Iglesias<sup>1</sup>, Gastón Iturburu<sup>3</sup>, Mirta Menone<sup>3</sup>, Martín Eguaras<sup>1</sup>, Sandra Medici<sup>1</sup>

<sup>1</sup>Centro de investigación en abejas sociales (CIAS), Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Mar del Plata (UNMDP). Centro de asociación simple CIC PBA. Mar del Plata, Buenos Aires, Argentina.

<sup>2</sup>Fares Taie Biotecnología Mar del Plata, Argentina

<sup>3</sup>Instituto de Investigaciones Marinas y Costeras. CONICET. Mar del Plata-

Agrochemicals, and particularly insecticides in sublethal doses, are widely recognized as being among the group of stressors that drastically reduce the fitness of insects. Particularly, *Apis mellifera* is known for its association with agriculture to sublethal contact with a wide range of this group of stressors that trigger different types of stress, such as nutritional stress. Based on this, the aim of this research is to determine if the level of total body proteins and the expression of the Vitellogenin gene in three days emerged bees varies before the topical exposure to a sublethal dose (LD10) of the pesticides Imidacloprid, Bifenthrin and Chlorpyrifos or a combination of pairs of them. The bioassay was performed on a total of 30 *A. mellifera* workers per treatment, which were contaminated with each pesticide or combination of them and after 24 hours were sacrificed with liquid nitrogen. Total protein content was obtained according to Bradford (1976) while Vitellogenin mRNA levels were determined by RT-qPCR. No significant differences were observed in the total protein content between the control group and the individual treatment groups with bifenthrin and chlorpyrifos, however in the rest of the treatments the total protein content was significantly lower than the control. A significant difference in mRNA levels was also found between individuals in the untreated group and all of the treated groups. Within the treated groups, the mRNA expression in the group in contact with bifenthrin was significantly higher than among all other groups. It was concluded that the individuals that were in contact with the agrochemicals studied presented a lower synthesis of vitellogenin mRNA, which may coincide with studies in other insect species. While it was postulated that this type of agrochemicals to a greater or lesser degree, depending on the family stimulates the corpora allata generating an increase in the juvenile hormone and therefore, in the case of bees, an inhibition of the synthesis of vitellogenin.





## Bee Health

### PP-284 [Bee Health]

#### “Varroa destructor” in an apiary in the municipality of Petrolina – PE, Brazil

Francisco Israel Lopes Sousa, Marileide De Souza Sá, Lucas Eugênio Pereira Babosa, Lucas Moura

Ribeiro Afonso, Catiana Da Conceição Vieira Melquiades, Eva Monica Sarmiento Da Silva

Department of Zootechnics, Federal University of São Francisco Valley, Petrolina, Pernambuco, Brazil

The mite “Varroa destructor” has been developing in recent years in several Brazilian apiaries, which may be associated with the abandonment and loss of colonies, leading to decreased production and productivity for beekeepers. In view of the above, the aim of this study was to verify the occurrence of “Varroa destructor” mite in an apiary in the municipality of Petrolina-PE. The experiment was carried out from 03/20 to 03/24/23, with two methods of analysis (adult bees and closed brood cells), in five random boxes (CX- 1;2;3;4;5). The adult bees (120) were collected in plastic tanks with 70% alcohol and the brood cells were taken from the brood frame itself (100 broods), packed in plastic bags, sealed and sent to LEAPMEL (Laboratory of Entomology, Apiculture and Meliponiculture), to check the amount of bees and mites found. The data were analyzed by variance analysis, in the SISVAR program, for the Tukey test at 5% significance level. The occurrence of “Varroa destructor” was verified in both analyzed methods. However, in the brood cells obtained a greater quantity and diversity of the mites (males and females (in various stages: young and adult). Observing the occurrence among the boxes in the adult bees, it was verified a higher occurrence in box 4 (Me=5'2), while boxes 1 and 2 presented a better performance (Me=0). Regarding brood cells, boxes 2 and 4 presented the worst performance (Me=17.9 and 28.3) respectively, box 5 presented the best performance (Me=0). We conclude that the area with brood cells presents a better performance for determining the infestation rate of “Varroa destructor” and hygiene in apiaries, thus avoiding a decrease in productivity.

### PP-285

#### Varroa destructor infestation in adult bees of Apis mellifera in Pantanal, Brazil, 2018-2021

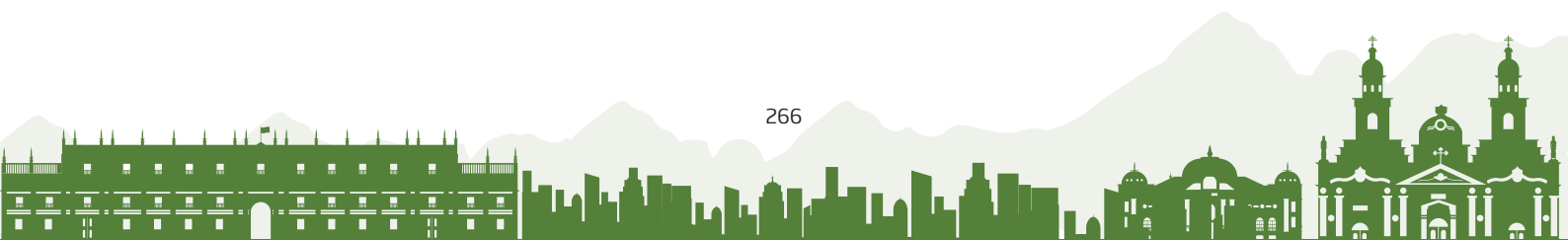
Vanderlei Doniseti Acassio Dos Reis<sup>1</sup>, Vanderlei Doniseti Acassio Dos Reis<sup>2</sup>, Simone França Lemes Fernandes<sup>3</sup>, Carlos Adriano Ojeda Salles<sup>3</sup>

<sup>1</sup>Embrapa Pantanal (Brazilian Agricultural Research Corporation/Pantanal)

<sup>2</sup>Nuclear Energy Center for Agriculture, University of São Paulo (CENA/USP)

<sup>3</sup>Biological Sciences

Brazil has a high potential for beekeeping production and is considered one of the exporters of high-quality honey. However, it ranked eleventh in world honey production and had only 4.8% of global export capacity in 2019. In addition, parasitism caused by the mite *Varroa destructor*, a virus vector and ectoparasite of larvae and adult bees of *Apis mellifera* still lacks further studies in the country. This work aimed to determine the infestation rate of this ectoparasite in the two castes of adult bees: workers and drones. In the analysis of the adult bees, five colonies were used in an Embrapa Pantanal apiary located on the Nhumirim ranch (18°59'15.70”S, 56°37'09.30”W), sub-region of Nhecolândia of Pantanal, in Corumbá-MS, Brazil. Collections were carried out between October 2018 and March 2021. The number of ectoparasites in adult bees was analyzed after removing approximately 100 bees from each colony. The infestation rate was reached by the formula: infestation rate (%) = (number of mites/number of bees) x 100. The months of analyzes were divided according to the seasons of the year, spring: October, November and December; summer: January, February and March; autumn: April, May and June, and winter: July, August and September, it should be noted that the first day of each month was adopted as the beginning of these seasons. The acquired results were expressed as mean (%) and standard deviation. The highest infestation occurred in the spring (April) of 2020 with an average of  $10.78 \pm 5.36$  and the lowest also in the spring (June) of 2020 with an average of  $1.42 \pm 1.64$ . According to the results of this work, the rates of infestation by *V. destructor* are within the parameters determined in Brazil based on other authors, relating the situation of Africanized bees with the subspecies of other countries. However, as there is little bibliographic data for places where the climate is tropical, as well as the Pantanal region, more long-term research is needed to monitor variations in infestation levels and possible damage to Africanized bees caused by the mite *V. destructor*.



### PP-286

### Evaluation Of The Therapeutic Efficacy Of Two Essential Oils *Laureliopsis Philippiana* And *Laurelia Sempervirens* For The Sanitary Control Of *Varroa Destructor* Anderson And Trueman) In *Apis Mellifera*

**Karina Uribe<sup>1</sup>, Carlos Lüders<sup>2</sup>, José Becerra<sup>3</sup>, Francisco Ramon Encina Montoya<sup>4</sup>**

<sup>1</sup>Departamento de Ciencias ambientales y acuícolas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile.

<sup>2</sup>Departamento de Ciencias Veterinarias, Universidad Católica de Temuco, Temuco, Chile.

<sup>3</sup>Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile.

<sup>4</sup>Departamento de Ciencias Ambientales, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile.

It is known that bees are not exempt from diseases that reduce honey production, which leads to the importance of hive health in production management. Among the most prevalent diseases that must be reported to the SAG in Chile is varroasis, a disease caused by the ectoparasite *Varroa destructor*. Beekeepers use a wide range of acaricide drugs to deal with this parasite. However, the incorrect or repeated application results in low therapeutic efficacy and multiple cases of resistance. That is why in this constant search for new therapeutic options that are effective against varroa and environmentally friendly we have evaluated two essential oils from native tree species of Chile, namely: *Laurelia sempervirens* and *Laureliopsis philippiana*. **METHODOLOGY:** the water vapor entrainment method was used to obtain the essential oils and the chemical composition was determined by gas chromatography coupled to GC/MS mass spectrometry. Subsequently, the acute toxicity in bees and varroa was evaluated by contact and fumigation using the impregnated slat method, the therapeutic window (LC50 and LD50) was determined and the ecological risk assessment (QR) was carried out to be evaluated in field conditions. **RESULTS:** The main components of *L. sempervirens* is Safrole with 92.59% and for *L. philippiana* safrole (45.76%) and  $\beta$ -Linalool (32.54). Impregnated slats were the least toxic method for bees with a LD50 of 3.75  $\mu\text{g}/\text{mL}$  and in varroa of 1.94  $\mu\text{g}/\text{mL}$ . Indicating a therapeutic window of 0 and the QR is 3.01, i.e. the ecological risk is moderate. Finally showing in field conditions a promising effect for the control of *V. destructor* in comparison to the control treatment. Suggesting a new study to determine the synergistic effect of the essential oil.

### PP-287

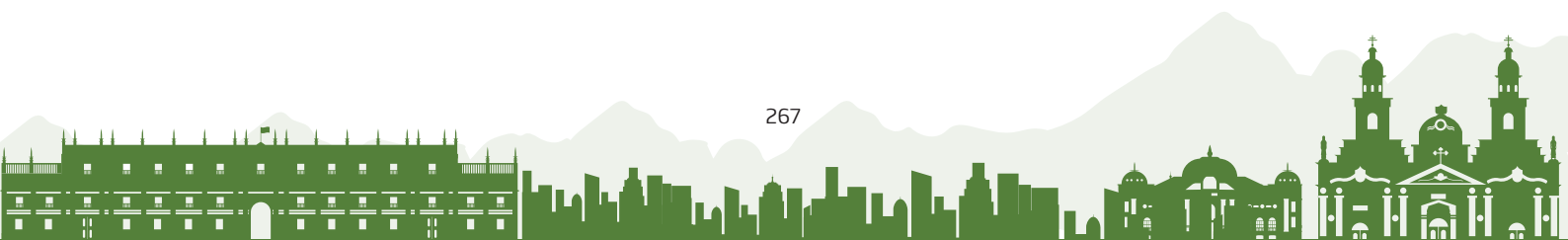
### Oxidative stress in nursing honey bees from colonies with different feeding management and exposed to a triple action fungicide

**Thaís Regina Ramos Alves<sup>1</sup>, Yara Martins Molina Ferraz<sup>1</sup>, Cássia Regina de Avelar Gomes<sup>1</sup>, Tainá Angelica De Lima Freitas<sup>1</sup>, Aline Yukari Kato<sup>1</sup>, Fábio Erminio Mingatto<sup>2</sup>, Daniel Nicodemo<sup>2</sup>**

<sup>1</sup>Post Graduate Program in Animal Science, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil.

<sup>2</sup>Department of Animal Science, São Paulo State University (Unesp), College of Agricultural and Technological Sciences, Dracena, Brazil.

A new strategy for combating phytopathogenic fungi is based on the use of fungicides with two or more active ingredients, aiming to control the population of fungi in different life stages simultaneously. If this alternative has been shown to be promising from an agronomic point of view, for beekeepers such a measure appears to be worrying. In this context, the nutritional status of the colonies must be considered, given that nutritional deficiencies can lead to greater susceptibility to stressors, such as fungicides. The objective of this work was to study the effects of food restriction and a commercial fungicide based on bixafen, trifloxystrobin and prothioconazole on the cellular antioxidant system of honey bees and the potential antioxidant action resulting from the food supplementation of the bee diet. For 15 weeks, 10 beehives from the same apiary were divided into two experimental groups. In the first group, five colonies had their natural food supplemented weekly with protein paste based on pollen and honey (4:1) and water and sugar syrup (1:1). The five colonies in the other group did not have their food supplemented and to restrict protein intake, a pollen collector was installed at the entrance of each hive. Newly emerged bees were obtained from these hives. They remained in cages for three days and were fed with sunflower pollen treated or not with Fox® Xpro fungicide and syrup based on water and sugar. The oxidative status was observed using muscle pools from the thorax of the bees. The activity of glutathione peroxidase (GPx) was higher in bees from colonies that received food supplementation, while catalase (CAT) and reduced glutathione activities (GSH) were higher in bees from colonies with restricted feeding. Pyridine nucleotides (NAD(P)H) concentration in the homogenate of the thorax was higher in bees from colonies that received food supplementation. CAT and GPx activities were higher when bees received pollen not treated with fungicide, respectively for colonies with supplemented and restricted diets. Nutritional management interfered in the activity of antioxidant enzymes and parameters related to the oxidative stress.



### PP-288

## Living together with American Foulbrood: long-term monitoring of *Paenibacillus larvae* in Uruguay

Sofia Palacios<sup>1</sup>, Daniela Arredondo<sup>1</sup>, Guillermo Añon<sup>1</sup>, Matilde Alarcón<sup>1</sup>, Pablo Zunino<sup>1</sup>, Juan Campá<sup>2</sup>, Jorge Harriet<sup>2</sup>, Karina Antúnez<sup>1</sup>

<sup>1</sup>Laboratorio de Microbiología y Salud de las Abejas, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay.

<sup>2</sup>Sección Apicultura, DILAVE, Ministerio de Ganadería, Agricultura y Pesca (MGAP), Uruguay

*Paenibacillus larvae* is the causative agent of American Foulbrood (AFB), the most severe bacterial disease affecting honey bees. It was reported in Uruguay in 1999 and since then, researchers and technicians from different institutions have worked together to monitor the spread of the bacterium. Here, we aim to share the Uruguayan experience. Two sampling strategies have been conducted. Firstly, in 2001/02, 2011, and 2021, we conducted nationwide studies collecting about 100 honey samples from colonies representing all Uruguayan provinces. The second strategy consisted of analyzing honey from storage tanks to be exported. Tanks were composed of honey (300 Kg) from multiple colonies in the same province. Between 2014-2019, 24-34 tank samples representing all Uruguayan provinces were evaluated per year. In 2001/02, the bacterium was widely distributed around the country, with a prevalence of 51%. Then, MGAP started a strong campaign to prevent its spread. Recommendations included vigilance for early diagnosis, elimination of colonies with symptoms, isolation and sterilization of materials in contact with affected colonies, and avoidance of using honey for bee feeding. The use of ethanolic extract of propolis was also suggested as a prevention method. Notably, the use of antibiotics was not allowed. These measures were successful, as in 2011, the prevalence of *P. larvae* in honey decreased to 2.0%. During the following years, monitoring based on honey tanks showed a slow increase in prevalence, from 3.6% in 2014 to 17.9 in 2019. The nationwide survey carried out in 2021, based on honey collected from colonies, confirmed the increase in the prevalence, reaching 11%. Nationwide surveys demonstrated the utility for monitoring the prevalence and distribution of pathogens. However, they are laborious and time-consuming. On the other hand, monitoring using honey from tanks may be a helpful alternative in countries with low AFB incidence, in big countries or regions, to facilitate representative samplings, and in countries where *P. larvae* has not been detected yet. Uruguay has been living with AFB for almost 25 years, but no severe outbreaks have been reported in many years, so our experience may be valuable for other countries.

### PP-289

## Study on the Effect of Feed Additive "Fanfengling" on Honeybee Autumn Reproduction

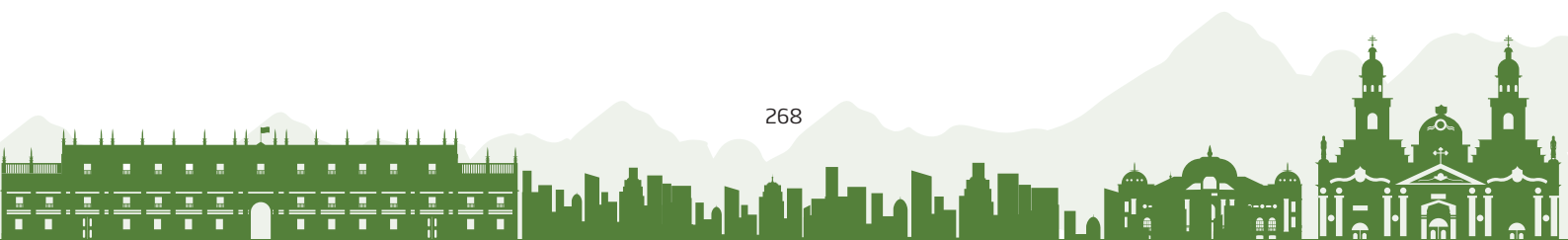
Guo Yuan<sup>1</sup>, Shao You Guan<sup>1</sup>, Zhang Long<sup>2</sup>, Zhao Meng<sup>3</sup>

<sup>1</sup>College of Horticulture, Shanxi Agricultural University, Shanxi Taiyuan 030801, China

<sup>2</sup>Fengying professional apiculture cooperative, Yanhu District West Street, Shanxi Yuncheng, 044000, China

<sup>3</sup>Shanxi WeiPeng Pharmaceutical Co., Ltd., Yuncheng, Shanxi, 044000, China

Bee colony breeding in autumn is an important part of beekeeping technology, taking into account both the breeding of collecting bees and the cultivation of overwintering bees of the right age. The quantity and quality of bee reproduction in autumn determine the success or failure of the next spring reproduction. The quality of feed plays a vital role in the whole autumn propagation. As a kind of bee feed, feed additives ensure the nutrients needed for the growth and development of bees. "Fanfengling" as one of the nutritional feed additives, in order to investigate the application effect of the bee feed additive "Fanfengling", "Fanfengling" was added to "Songdan No.1" bees during the breeding season in the autumn. The results showed that when bees were fed "Fanfengling", the number of eggs and pupae increased by 24.07%, the net weight of bees increased by 50.6%, the pollen yield increased by 13.92%, the survival rate of bees overwintering increased by 27.22%, and the weight of overwintering bees increased by 36.81% compared with the control group, indicating that feeding the bee colony with "Fanfengling" can significantly improve bees' reproduction, production and overwintering ability.



### PP-290

#### Proteomic and Metalloproteomic as tool to Analisis of the Royal Jelly Produced By *Apis Mellifera L.* Bees

Ricardo De Oliveira Orsi, Adriana Fava Negrão, Pedro Magalhães Padilha

Center of Education, Science and Technology in Rational Beekeeping (NECTAR), College of Veterinary Medicine and Animal Sciences, UNESP Sao Paulo State University, Sao Paulo, Botucatu, Brazil

Royal jelly is a secretion of the mandibular and hypopharyngeal glands of works nursing bees. This bee product has a complex composition of proteins, carbohydrates, vitamins, lipids and minerals. Minerals like zinc and iron are fundamental, acting as enzymatic cofactor, participating in metabolism, regulation of gene expression, structural maintenance of biomembranes, immunity and protection against free radicals. However, its presence depends on the floral resource available, which may vary throughout the year. Therefore, studies that analyze the royal jelly are extremely important, serving as a tool to help in understanding the nutrition of colonies. This study analyzed the protein content, number of protein spots and the content of minerals (Zn and Fe) found in the royal jelly in different seasons. Royal jelly was collected during the period from January to December, by Doolittle method. The samples were stored in sterile vials and the protein content determined by the Biuret method. Two-dimensional electrophoresis for the fractionation of royal jelly proteins was performed and identified by electrospray ionization mass spectrometry (ESI MS MS). Zinc and iron level was determinate by the flame atomic absorption spectrometry (FAAS). The results evaluated were by ANOVA followed by Tukey's test to check for differences between means ( $P < 0.05$ ). The results do not showed differences in the proteins contents of the royal jelly in the seasons (spring  $3.91 \pm 0.36\%$ ; summer  $3.89 \pm 0.40\%$ ; autumn  $3.40 \pm 0.07\%$  and winter  $3.33 \pm 0.27\%$ ). However, the number of spots was major in the autumn ( $106.6 \pm 18.8$ ) when compared to the summer ( $72.42 \pm 11.2$ ), do not differ from the spring ( $85.6 \pm 24.6$ ) and winter ( $95.30 \pm 23.7$ ). Zn was the major mineral found of analyzed spots compared to Fe throughout the year. In some months (February to June and September) these minerals were observed simultaneously in the spots. The proteins more abundant was the MRJP2, followed by MRJP3 and MRJP1 (important by playing nutritional functions), besides of the proteins MRJP5, MRJP6, MRJP7 and MRJP9. The results show that the seasons modified the royal jelly protein composition and the possible interactions with metals such as zinc and iron, being the proteomic and metalloproteomic as an important tool to study the honeybee's nutrition.

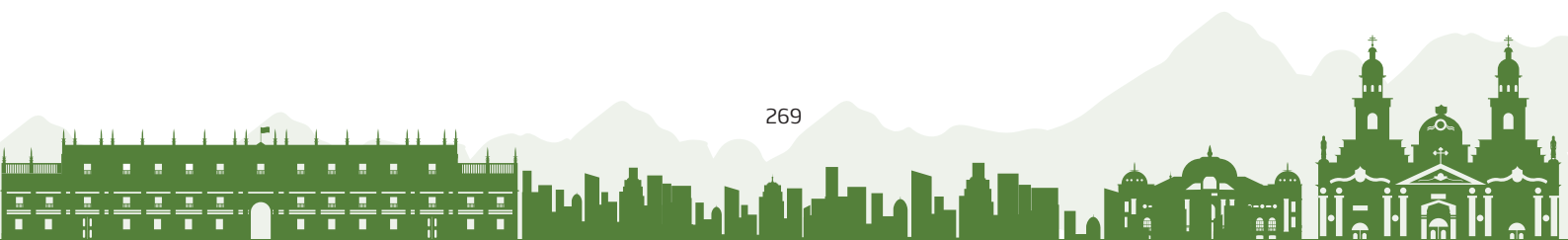
### PP-291

#### Supplementation with an Inorganic and Organiz Zinc Source in the Metalloproteomic Profile of Royal Jelly in *Apis mellifera L.*

Ricardo De Oliveira Orsi, Aimê De Almeida Longuini, Marcelo Polizel Camilli, Pedro Magalhães Padilha

Center of Education, Science and Technology in Rational Beekeeping (NECTAR), College of Veterinary Medicine and Animal Sciences, UNESP Sao Paulo State University, Sao Paulo, Botucatu, Brazil

Royal Jelly is an important food to young larvae and queen of the *Apis mellifera* honeybees. In its composition, the mineral contents could be varied throughout the year because the floral resource available to the bees. Zinc is a mineral can act as as enzymatic cofactor, regulate of gene expression, structural maintenance of biomembranes, and protection against free radicals, among others. However, in some seasons its contents could be reduced in the honeybee's resources, affecting the royal jelly quality. So, this study aimed to supplement the colonies of *A. mellifera* with different concentrations of inorganic (Zinc Sulphate monohydrate) or organic (zinc-methionine) zinc (0, 25, 50 and 75 mg mL<sup>-1</sup>) and evaluate protein expression of the royal jelly. Were performed two-dimensional electrophoresis and the proteins identified by electrospray ionization mass spectrometry (ESI MS MS). Flame atomic absorption spectrometry (FAAS) was used to determinate the zinc level. Tukey test ( $P < 0.05$ ) was used to analyses the results. Inorganic zinc (IZn) positively affected the quantification of proteins for treatments 50 and 75 mg mL<sup>-1</sup>. However, all treatments independent of IZn concentrations showed fewer protein spots when compared to the control. All proteins were classified as Major Royal Jelly Proteins (MRJPs). The exposure of nursing bees to IZn reduced the expression of six different MRJP proteins involved in larval development and glands of nursing bees (MRJP1, MRJP2, MRJP3, MRJP5 and MRJP7); however promoted an increase in the proteins involved in defense systems (MRJP8 and MRJP9). Organic zinc-containing (OZn) diets modified total royal jelly protein content and altered the expression of five major royal jelly proteins (MRJP1, MRJP2, MRJP3, MRJP5, and MRJP8). While the diet with the lowest tested OZn content (25mg mL<sup>-1</sup>) increased total royal jelly protein content and expression of MRJP1, diets with higher OZn contents (50 and 75mg mL<sup>-1</sup>) decreased MRJP expression. The results demonstrate that vital proteins and metabolic processes are affected in nursing bees exposed to the mineral zinc. Organic zinc source in lower concentrations increase the protein contents in the royal jelly probably due to its major available. Proteomic and metalloproteomic is an important tool to study the honeybee's nutrition.





### PP-292

### Finger print profiling of Colombian propolis samples using: HPTLC, UV, FT-IR, 1H-NMR and 13C-NMR

Guillermo Salamanca Grosso, Monica Osorio, Anderson Guarnizo Franco

Mellitopalinalogical Research Group, Department of Chemistry, Faculty of Sciences. University of Tolima

Propolis, is known as the bee glue, combines resins collected by the honey bees from different plant organs, and with beeswax that honey bees additionally incorporate. Chromatographic or spectroscopic fingerprint profiling is one of the most promising tools routinely used in raw material standardization. The present study aimed to investigate the finger print propolis from different regions of Colombia (CP). Chemical profiling of ethanolic extracts (EECP), was investigated using HPTLC, UV, FT-IR, 1H-NMR and 13C-NMR techniques. Extracts (3  $\mu$ L) were applied to the 20  $\times$  10 cm. HPTLC plates as 5 mm. Development was performed toluene-ethyl acetate-formic acid (30:12:5, P' 3.39). Infrared spectroscopy was performed using FT-IR analysis in the region of (4000-650)  $\text{cm}^{-1}$ . A broad strong band at 3350  $\text{cm}^{-1}$  observed in analyzed propolis samples occurs due to the O-H stretching vibration of the phenolic group. Spectral features related to phenols are also characterized by interaction of O-H deformation and C-O stretching vibrations which can be observed in the spectral range between 1405 and 1220  $\text{cm}^{-1}$ . Phenols are also represented with a doublet at 1640  $\text{cm}^{-1}$  assigned to aromatic ring C=C stretching and aromatic C-H deformation vibration at 1110  $\text{cm}^{-1}$ . The most prominent absorption in the fingerprint region is a broad band observed at 1170  $\text{cm}^{-1}$  that corresponds to the C-O asymmetric stretching vibration of esters related to long-chain aliphatic acids. Inspection of the 1H-NMR spectra allows one to obtain information regarding chemical heterogeneity of the samples that revealed the presence of different phytochemical compounds based on peaks in specific regions. The peaks occurred in the region 0.5–3.0 ppm, and were mainly terpenes, whereas peaks found in spectrum regions 3.5–6.0 and 5.5–8.00 ppm were sugars and phenolics, respectively. 13C-NMR show specific groups as acetyl and the ester groups due to the presence of signals in the area between 160 and 170 ppm. This is the first work that relates the general properties of Colombian propolis

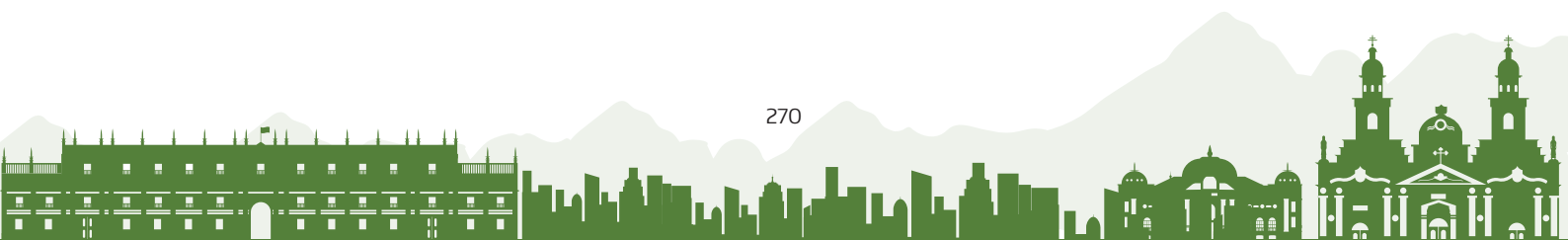
### PP-293

### Botanical origin of honeys from the area of influence of Tame, Arauca, Colombia

Guillermo Salamanca Grosso, Monica Osorio, Estefanía Hernández Muñoz, Oscar Julian Vanegas Florez

CMellitopalinalogical Research Group, Department of Chemistry, Faculty of Sciences. University of Tolima

The study area is located in the southwest of the department of Arauca, in the municipality of Tame, between the geographical coordinates 6° 24' 34,604", 6° 19' 8,274" north latitude and 71° 53' 41,493", -72° 1' 48,955" west latitude, in the Tame municipality. latitude -72° 1' 48,955" west, in the foothills of the Eastern Cordillera. The climate is tropical humid and dry according to the Koppen system, where there is a bimodal system of rains (April to November) and drought (December to March). Average precipitation is close to 2800 mm/year, thermal sensations, reaching 26 °C with maximums between March and April. Relative humidity is around 82% during the rainy season and 70 to 75% during the dry season. The related areas correspond to the life zones of humid and very humid premontane forest (bh-PM; bmh-PM), humid and very humid tropical forest (bh-T; bmh-T). Selected samples of honey from the indicated life zones were acetolyzed and evaluated under the microscope, with subsequent estimation of the Dominance, Margalef, Shannon and Simpson indices. The results of the study allowed the identification of botanical resources used by *Apis mellifera* L., from colonies established in the bee fields of Tame. Forty pollen types belonging to 22 botanical families were determined, including *Solanum* sp., *Hymenaea courbaril* L. and *Psidium guajava* L.; *Ricinus communis* L., *Calophyllum brasiliense* Bromelia sp., *Mimosa* sp. and *Rynchospora nervosa* (Vahl) Boeckeler, *Myrcia* sp., *Davilla kunthii* A.St.-Hil. and *Clusia grandiflora* Splitg. *Mangifera indica* L., *Handroanthus chrysanthus* (Jacq.) S.O. Grose and *Tapirira guianensis* Aubl, *Anacardium excelsum* (Kunth) Skeels, *Mimosa* sp. and *Hymenaea courbaril* L., *Warszewiczia coccinea* (Vahl) Klotzsch; *Vismia guianensis* (Aubl.) Pers and *Handroanthus chrysanthus* (Jacq.) S.O.Grose A. mellifera, show specific preference patterns. This work is a contribution to the melisopalynological study of Colombian honeys, consolidating as the first report of paleobotanical markers of honeys from Arauca.





### PP-294

### Perspectives for the use of ethanolic bee-pollen extracts as a biopreservative to inhibit the growth of fungi in bakery products

Alejandra Castillo Arias<sup>1</sup>, Carlos Alberto Fuenmayor<sup>2</sup>, Carlos Zuluaga Dominguez<sup>3</sup>

<sup>1</sup>Universidad Nacional de Colombia –Sede Bogotá – Programa de Doctorado en Ciencia y Tecnología de Alimentos – Carrera 30 # 45-03 Edificio 500A, Bogotá D.C, 111321 -Colombia

<sup>2</sup>Universidad Nacional de Colombia –Sede Bogotá –Instituto de Ciencia y Tecnología de Alimentos–Carrera 30 # 45-03 Edificio 500A, Bogotá D.C, 111321 –Colombia.

<sup>3</sup>Universidad Nacional de Colombia –Sede Bogotá –Facultad de Ciencias Agrarias –Departamento de Desarrollo Rural y Agroalimentario –Carrera 30 # 45-03 Edificio 500, Bogotá D.C, 111321 –Colombia

Bee-pollen is a highly appreciated product thanks to the richness of its composition in substances with nutritional and functional qualities. Several classes of bioactive compounds, including carotenoids, flavonoids, and other phenolics have been demonstrated to act as antimicrobial, antifungal, antioxidant, anticancer, and anti-inflammatory agents. In response to current consumer trends, that favor more natural products, there is an increasing interest in the use of bee pollen and bee pollen extracts as sources of natural additives for the food industry. Food spoilage is a concern for the industry, as it can lead to food waste, economic losses for manufacturers and consumers, and a negative impact on food brands. Particularly in bakery products, the development of fungi can lead to visual deterioration, smell, flavor, and texture, as well as lead to negative impacts on health through the production of mycotoxins. The baking industry typically uses compounds such as calcium propionate to inhibit fungal growth. However, the presence of this synthetic additive usually has a negative impact in terms of consumer perception and preference, as it has been associated with carcinogenic effects. Due to the presence of phenolic and other bioactive compounds, ethanolic bee-pollen extracts are potential additives to replace artificial preservatives. Consequently, this work aimed to determine the effectiveness of bee-pollen extracts as a food biopreservative. Dried and defatted bee-pollen samples were taken and subjected to an extraction process with 96% ethanol for the extraction of bioactive compounds with continuous stirring (500 rpm) for 24 h. Then, the supernatant was separated and centrifuged at 4000 rpm for 10 minutes, filtered with qualitative filter paper, and rotaevaporated for 30 minutes at 45°C. This concentrated extract was tested for minimum inhibitory concentration against *Aspergillus* sp., commonly found in spoiled bakery products, and antioxidant activity against DPPH and ABTS+ radicals. A concentration as low as 1.25 µl/ml was able to inhibit the growth of these fungi, which in addition to its antioxidant activity indicates its potential as a natural preservative agent. These results are the rationale for developing new bakery products and other foodstuffs with the use of affordable natural additives linked to healthy lifestyles.

### PP-295

### Authenticity evaluation of Colombian honey by electronic tongue and electronic nose

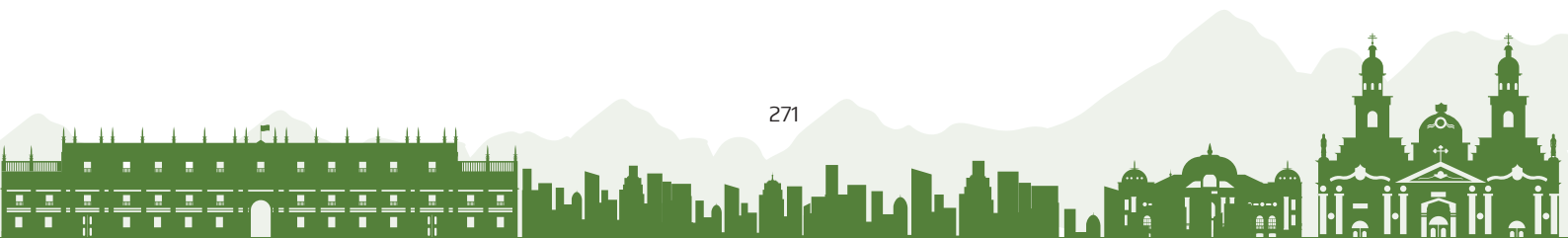
Diana Acosta Opayome<sup>1</sup>, Carlos Alberto Fuenmayor<sup>2</sup>, Carlos Zuluaga Domínguez<sup>3</sup>

<sup>1</sup>Universidad Nacional de Colombia –Sede Bogotá – Programa de Maestría en Ciencia y Tecnología de Alimentos – Carrera 30 # 45-03 Edificio 500A, Bogotá D.C, 111321 -Colombia

<sup>2</sup>Universidad Nacional de Colombia –Sede Bogotá –Instituto de Ciencia y Tecnología de Alimentos–Carrera 30 # 45-03 Edificio 500A, Bogotá D.C, 111321 –Colombia

<sup>3</sup>Universidad Nacional de Colombia –Sede Bogotá –Facultad de Ciencias Agrarias –Departamento de Desarrollo Rural y Agroalimentario –Carrera 30 # 45-03 Edificio 500, Bogotá D.C, 111321 –Colombia

Sensory evaluation is an activity based on the perception of the senses of sight, smell, taste, touch, and hearing for the characterization, differentiation, description, and classification of foods. As an analysis tool, it can be very complex due to the subjectivity of the responses from panelists. In this context, the so-called instrumental sensory analysis techniques are of great interest since they use physicochemical principles to determine chemical characteristics directly or indirectly related to sensory attributes. In the particular case of beehive products, such as honey, sensory attributes are a fundamental part of their overall quality. Thus, instrumental sensory analysis techniques, such as electronic noses and tongues, have been combined with chemometrics to obtain relevant sensory information, in a more objective way and through easy-to-use instruments, compared to a traditional sensory panel. Electronic nose and tongue have been proven to be fast, inexpensive, and reliable tools to confirm that a particular honey sample belongs to a specific floral origin. In this work, 115 honey samples from different geographical origins from Colombia were analyzed with an electronic nose which contained 10 metal oxide sensors, and a voltammetric electronic tongue made up of three screen-printed electrochemical sensors: gold, platinum, and graphite. In addition, to evaluate the voltammetric electronic tongue as an authentication tool, 50 adulterated samples were analyzed by both techniques, and the resulting signals were compared to those of 50 authentic honey samples. Honey samples were not differentiated according to geographical origin by the electronic nose and tongue, however, the Principal Component Analysis of the electronic tongue responses, differentiated adulterated from authentic honey. The method to evaluate honey authenticity proposed in this work can be used as a complementary tool to the traditional analytical methods used to establish food adulteration and commercial fraud in foodstuff as a guarantee of protection for consumers.



### PP-296

#### Advances in classification and differentiation of Colombian honey through microbiological and chemical indicators, electrochemical sensors, and chemometrics

Alejandra Castillo Arias<sup>1</sup>, Carlos Alberto Fuenmayor<sup>2</sup>, Carlos Zuluaga Dominguez<sup>3</sup>

<sup>1</sup>Universidad Nacional de Colombia –Sede Bogotá – Programa de Doctorado en Ciencia y Tecnología de Alimentos – Carrera 30 # 45-03 Edificio 500A, Bogotá D.C, 111321 -Colombia

<sup>2</sup>Universidad Nacional de Colombia –Sede Bogotá –Instituto de Ciencia y Tecnología de Alimentos–Carrera 30 # 45-03 Edificio 500A, Bogotá D.C, 111321 –Colombia.

<sup>3</sup>Universidad Nacional de Colombia –Sede Bogotá –Facultad de Ciencias Agrarias –Departamento de Desarrollo Rural y Agroalimentario –Carrera 30 # 45-03 Edificio 500, Bogotá D.C, 111321 –Colombia

Bee-pollen is a highly appreciated product thanks to the richness of its composition in substances with nutritional and functional qualities. Several classes of bioactive compounds, including carotenoids, flavonoids, and other phenolics have been demonstrated to act as antimicrobial, antifungal, antioxidant, anticancer, and anti-inflammatory agents. In response to current consumer trends, that favor more natural products, there is an increasing interest in the use of bee pollen and bee pollen extracts as sources of natural additives for the food industry. Food spoilage is a concern for the industry, as it can lead to food waste, economic losses for manufacturers and consumers, and a negative impact on food brands. Particularly in bakery products, the development of fungi can lead to visual deterioration, smell, flavor, and texture, as well as lead to negative impacts on health through the production of mycotoxins. The baking industry typically uses compounds such as calcium propionate to inhibit fungal growth. However, the presence of this synthetic additive usually has a negative impact in terms of consumer perception and preference, as it has been associated with carcinogenic effects. Due to the presence of phenolic and other bioactive compounds, ethanolic bee-pollen extracts are potential additives to replace artificial preservatives. Consequently, this work aimed to determine the effectiveness of bee-pollen extracts as a food biopreservative. Dried and defatted bee-pollen samples were taken and subjected to an extraction process with 96% ethanol for the extraction of bioactive compounds with continuous stirring (500 rpm) for 24 h. Then, the supernatant was separated and centrifuged at 4000 rpm for 10 minutes, filtered with qualitative filter paper, and rotaevaporated for 30 minutes at 45°C. This concentrated extract was tested for minimum inhibitory concentration against *Aspergillus* sp., commonly found in spoiled bakery products, and antioxidant activity against DPPH and ABTS+ radicals. A concentration as low as 1.25 µl/ml was able to inhibit the growth of these fungi, which in addition to its antioxidant activity indicates its potential as a natural preservative agent. These results are the rationale for developing new bakery products and other foodstuffs with the use of affordable natural additives linked to healthy lifestyles.

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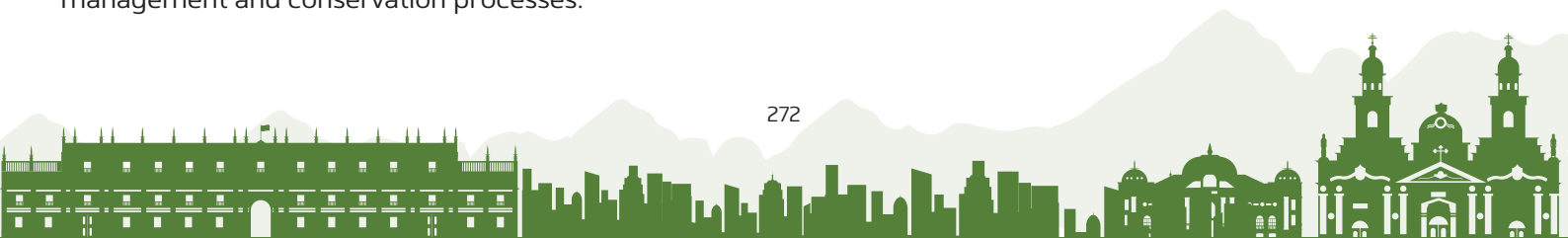
Carlos Alberto Fuenmayor<sup>1</sup>, Carlos Mario Zuluaga Domínguez<sup>2</sup>, Claudia Salazar González<sup>1</sup>, Andrés Niño Aparicio<sup>1</sup>, Nicolás Ariza Cruz<sup>3</sup>, Andrea Nieto Veloza<sup>1</sup>

<sup>1</sup>Universidad Nacional de Colombia –Sede Bogotá –Instituto de Ciencia y Tecnología de Alimentos (ICTA)

<sup>2</sup>Universidad Nacional de Colombia –Sede Bogotá –Facultad de Ciencias Agrarias –Departamento de Desarrollo Rural y Agroalimentario

<sup>3</sup>Universidad Nacional de Colombia –Sede Bogotá –Facultad de Ingeniería

The consumption of honey in Colombia is still relatively low compared to other countries, despite consumers having a high appreciation for this food. Some factors that may be associated with this reduced consumption are related to a yet little knowledge about the physicochemical, nutritional, and functional characteristics of these products, as well as problems in the beekeeping productive chain such as adulteration, and limited processes to preserve and adequate honey under parameters of quality and safety. In this work, the microbiological (aerobic mesophiles, molds and yeasts, *Escherichia coli* and *Salmonella*) and physicochemical quality characteristics (pH, acidity, moisture, minerals, sugars, hydroxymethylfurfural, electrical conductivity, and insoluble solids) of 60 honey samples from Colombia were analyzed with the objective to improve harvesting processes, generate added value and search differences that lead to the establishment of the Designation of Origin. The information collected through the previously mentioned analyses was complemented with instrumental techniques such as an electronic nose equipped with 10 metal oxide sensors as well as an electronic tongue equipped with three voltammetric sensors: graphite, platinum, and gold. The physicochemical composition was in accordance with the parameters referenced in the bibliography and complied with national and international regulations. The responses of sensors and the results obtained from the physical-chemical analysis were evaluated by Principal Component Analysis (PCA) and discriminant analysis (DA). The results showed that it was possible to differentiate and classify honey samples according to their origin. A correlation was found between physical-chemical results and data from the electronic nose and tongue, suggesting these are useful tools for the characterization and quality control of honey and that volatile and non-volatile compounds present in bee products contribute significantly to flavor variation, which in turn is related to botanical and geographical origin. These results will enable origin denomination labels for honey from these regions and the improvement in management and conservation processes.



### PP-296

### Advances in classification and differentiation of Colombian honey through microbiological and chemical indicators, electrochemical sensors, and chemometrics

Alejandra Castillo Arias<sup>1</sup>, Carlos Alberto Fuenmayor<sup>2</sup>, Carlos Zuluaga Dominguez<sup>3</sup>

<sup>1</sup>Universidad Nacional de Colombia –Sede Bogotá – Programa de Doctorado en Ciencia y Tecnología de Alimentos – Carrera 30 # 45-03 Edificio 500A, Bogotá D.C, 111321 -Colombia

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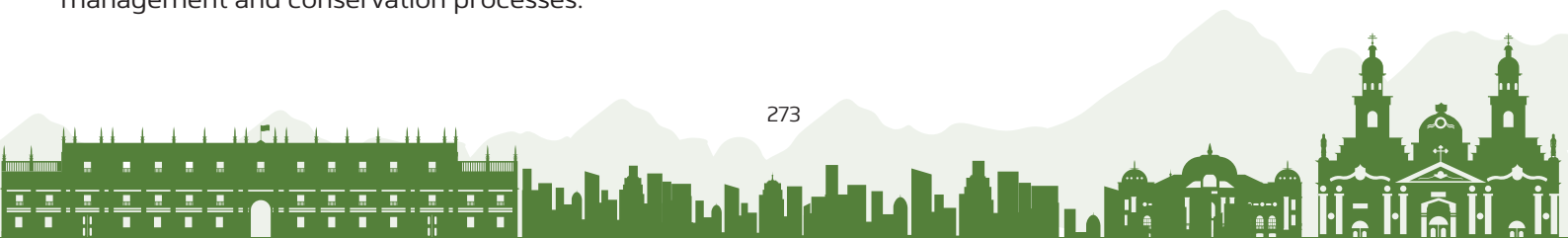
Carlos Alberto Fuenmayor<sup>1</sup>, Carlos Mario Zuluaga Domínguez<sup>2</sup>, Claudia Salazar González<sup>1</sup>, Andrés Niño Aparicio<sup>1</sup>, Nicolás Ariza Cruz<sup>3</sup>, Andrea Nieto Veloza<sup>1</sup>

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### PP-297

### Quillay honey: chemical characterization, antioxidant and hepatoprotective potential

Paula Nunez-Pizarro<sup>1</sup>, Gloria Montenegro<sup>2</sup>, Raquel Bridi<sup>3</sup>

<sup>1</sup>Doctorado en Ciencias de la Agricultura, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>2</sup>Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>3</sup>Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

Quillay (*Quillaja saponaria* Mol. (Quillajaceae)) is a Chilean endemic tree that grows in the central Mediterranean climate regions of the country, and it is one of the most important melliferous species in Chile, mainly as a source of unifloral honey.

The biological activity of this endemic honey is associated with the bioactive chemical compounds, like phenolics, from the nectar. The result is a natural food with great nutritional value and potential for therapeutic use.

This study aimed to establish a phytochemical screening of quillay honey evaluating (i) the botanical origin, (ii) the phenolic profile, (iii) the antioxidant capacity, (vi) the antimicrobial activity and (v) the hepatoprotective effects in HUH7 cells.

The botanical origin determined by palynological analysis showed that 9 out of 12 studied samples have more than 45% of pollen from *Q. Saponaria*, corresponding to unifloral honey. According to the findings, there was a large amount of phenolic and flavonoid content in the samples. They showed antioxidant ability and reducing power. All samples included the flavonoids chrysin and galangin as well as the acids chlorogenic, syringic, cumaric, and sinapic, according to the HPLC-DAD analysis. All samples showed antibacterial activity against *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Escherichia coli* and *Staphylococcus aureus*. The hepatoprotective effect of the extracts of quillay honey was evaluated by the oxidation of HUH-7 hepatic cells with the potent oxidant AAPH. The results showed a significant increase in cell viability in the presence of honey extracts compared with AAPH treatment alone, suggesting the protective effects of those extracts against oxidative stress AAPH-induced free radicals' accumulation in the hepatic cells.

This study showed that the total phenol and flavonoid content of unifloral quillay honey is equal or superior to other honey from Chile and outperformed the other Chilean samples in both tests, FRAP and ORAC.

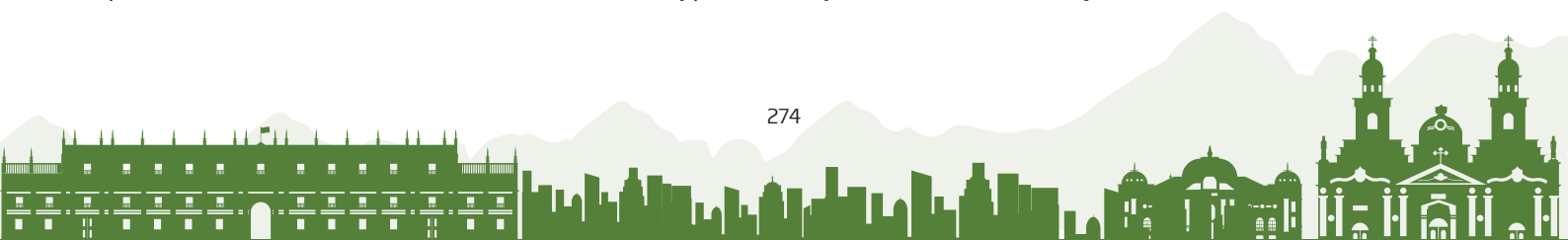
### PP-298

### Honeydew Honey Production in Fujairah, U.A.E

James Salome, Shaher Bano Mirza, Fouad Lamghari Ridouane

Fujairah Research Centre

The honey produced by the bees using excretions of plant-sucking insects is called honeydew honey. The main differentiating factors between blossom honey and honeydew honey are the sugar composition and electrical conductivity. The higher fructose contents and electrical conductivity makes honeydew honey better than other types of honey. Honeydew honey has a rich composition of nutrients in its content and can be a dietary supplement, and has therapeutic uses besides antioxidant and antimicrobial properties. Only some countries in the world, especially Turkey, Greece, Germany, Spain, and Bulgaria, have the necessary environmental conditions for producing this rare type of honey. Honey produced in the years 2018, 2019, 2020, and 2021 in different regions of the Emirate of Fujairah – UAE (Dibba, Fujairah, Ohala, Tawiyah) were sampled and sent to "German Laboratories" in Germany to check mainly fructose, glucose, invert sugar contents, and electrical conductivity. The fructose content in 10 samples produced in UAE presents the average value of  $36.4900 \text{ g/100g} \pm 1.7866$ . Similarly, the analysis of glucose contents in samples from Fujairah-UAE and other countries indicated similar average values calculated as  $27.2100 \text{ g/100g} \pm 4.5160$ . The inverted sugar contents indicate the average value for 10 samples as  $63.70 \text{ g/100g} \pm 6.2082$ . The electrical conductivity of samples from UAE showed an average value of  $1.4030 \text{ mS/cm} \pm 0,3528$ . The investigations showed the higher electrical conductivity reported for the honeydew honey sample produced (2019) in the Ohala desert, Fujairah- UAE, with a value of  $1.97 \text{ mS/cm}$ . Upon our investigation, the ten honey samples produced in different regions of the Emirate of Fujairah – UAE, can be considered honeydew honey, mainly by similar inverted sugar values and significantly higher levels of electrical conductivity than the samples studied in traditional producer countries such as Turkey, Greece, Poland, Bulgaria, Germany. However, none of the studies have been done so far specifically regarding the production of this differentiated and valuable type of honey in the Emirate of Fujairah – UAE.





### PP-299

#### Pollen Analysis of Honey Produced by *Apis dorsata* from Punjab, Pakistan

Samina Qamer, Muhammad Sajid

Department of Zoology, Government College University Faisalabad, Pakistan

Pollen analysis helped to categorize honey on the basis of its floral source. Fifty honey samples produced by *Apis dorsata* were collected from various botanical and geographical areas such as Multan, Jehlum, Chakwal, Murree, Kallar kahar and Rawalpindi of Punjab Province, and stored at room temperature (25 °C) in plastic jars. All the samples of honey were subjected to study of pollens, as per recommended method by International Honey Commission with the aim to confirm the floral origin of the honeys. Pollens count was carried out by haemocytometer under microscope which followed by statistical analysis. High quality pictures were taken by camera fitted on light microscope. Results showed that *Azadirachta* sp. (22.5%) pollen highest %age followed by *Ziziphus* sp. (13.9%), *Poacea* (13.6%), *Citrus* sp. (13.4%), *Acacia* sp. (10.2%), *Brassica* sp. (4.8%), *Eucalyptus* sp. (4.35%), *Moringa* sp. (3.46%), *Prosopis* sp. (3.4%), *Pisum* sp. (3.4%), *Syzygium* sp. (2.8%), *Psidium* sp. (1.2%), *Morus* sp. (1.2%), *Impatiens* sp. (1.2%), *Cajanus* sp. (0.6%), *Trifolium* sp. (0.6%) and *Pinus* sp. (0.5%) pollens were found in fresh honey samples.

### PP-300

#### Physicochemical and Antioxidant Characteristics of Honey from Mozambique

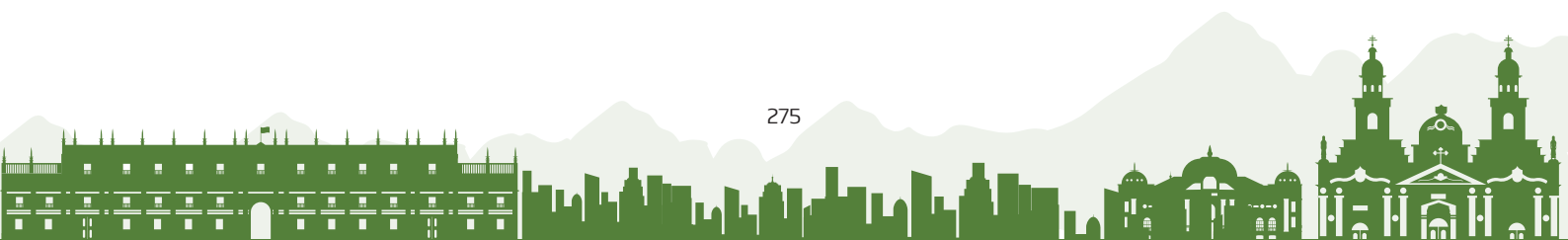
Fernando Joao Tanleque Alberto<sup>1</sup>, Porfirio Americo Nunes Rosa<sup>1</sup>, Isabel Escriche<sup>3</sup>, Deodete Chachuaio Chachuaio<sup>2</sup>

<sup>1</sup>Faculty of Food and Agricultural Sciences, Food Technology Department, Universidade Rovuma, Nampula, Mozambique.

<sup>2</sup>National Directorate of Local Economic Development, MADER, Mozambique.

<sup>3</sup>Institute of Food Engineering for Development (IUIAD), Food Technology Department (DTA), Universitat Politècnica de València, Valencia, Spain

Honey is one of the most primitive foods that exists, being highly valued for its nutritional and therapeutic properties, and especially for its antioxidant properties. The presence of this type of compounds and/or other physicochemical properties can be useful to enhance the value of honey from certain geographical areas, in addition to promoting the creation of new sustainable livelihoods in rural areas. Based on this, the aim was to characterise honey from different provinces of Mozambique in terms of their physicochemical quality parameters and antioxidant (specific flavonoids, phenolic acids profiles and the total antioxidant activity). Seventy honey samples from different provinces of Mozambique were analysed in the present study: 20 from Nampula, 15 from Zambezia, 15 from Manica, and 20 from Sofala. The methods of the International Honey Commission were applied to determine the physicochemical quality. The extraction of phenolic acids and flavonoid compounds was carried out submitting the honey samples to a solid-phase extraction and then analyzed using a HPLC-Alliance with a photodiode array detector. Seven phenolic acids (chlorogenic, caffeic, ellagic, ferulic, gallic, p-coumaric and sinapic) and eight flavonoids (catechin, chrysin, kaempferol, luteolin, naringenin, pinocembrin, quercetin and rutin) were screened in the samples. Nampula honey had a higher content of most of these compounds and the total antioxidant activity (even reaching up to 40mg TE/100g) compared honey from the other provinces. All the physicochemical parameters were in agreement with the international regulations. Most of the honey samples were classed as honeydew honey since they were dark and had conductivity values above 0.800 mS/cm.





### PP-301 Micromycetes on Bee Pollen

Aurelija Šaluchaitė, Jolanta Sinkevičienė, **Algirdas Amšiejus**  
Agriculture Academy of Vytautas Magnus University, Kaunas, Lithuania

Furthermore, favorable conditions that enable the synthesis of mycotoxins (adequate temperature, relative humidity, pH, and aw values) are found frequently during pollen collection and/or production process.

#### Materials and methods

The aim of the research was to determine the occurrence of microscopic fungi of the bee pollen treated by drying and UV radiation and the duration of storage. In June-July of 2021, a total of 36 pollen samples were removed from apiaries in central Lithuania. Bees have collected the pollen from different plants. The fresh pollen was dried in a drying-oven 12 hours, till humidity was 8%. The dilution method (CFU/g) was used to determine the number of fungi strains per sample and their amount. A series of dilutions (from  $10^{-1}$  to  $10^{-5}$ ) were prepared from the resulting suspension. Potato Dextrose Agar (PDA) medium was used in the study. After 5-7 days of incubation at 25°C in dark, the contamination level of samples by the different fungi was evaluated. All assays were performed in triplicate. Statistical analysis of data was performed by Anova.

#### Results

Table. Levels of fungi contamination in samples of bee pollen (cfu g<sup>-1</sup>)

Fungi Dried Dried+UV

Acromonium spp.  $2 \times 10^3$  -

Alternaria alternata (Fr.) Keissl.  $9 \times 10^3$   $5 \times 10^3$

Aspergillus flavus Link. -  $2 \times 10^2$

Botrytis spp.  $1 \times 10^3$   $6 \times 10^3$

Cladosporium cladosporioides  $3 \times 10^3$   $5 \times 10^3$

Fusarium sporotrichioides Sherb. -  $1 \times 10^2$

Mucor hiemalis Wehmer  $1 \times 10^3$   $1 \times 10^3$

Penicillium verrucosum Dierckx.  $4 \times 10^3$   $6 \times 10^3$

Rhizopus stolonifer (Ehrenb.) Vuill.  $7 \times 10^3$   $5 \times 10^3$

#### Conclusions

1. The amount of fungi in the dried and with UV-radiation treated pollen was averaged respectively 2.46 and 2.38 (cfu g<sup>-1</sup>).
2. Lowest fungi contamination (2.27 cfu g) established after 1 months stored pollen, treated with UV radiation and stored at 8°C.
3. After two months of storage, the contamination of fungi began to increase. Lowest fungi contamination established after 3 months stored pollen, treated with UV radiation and stored at 8°C.

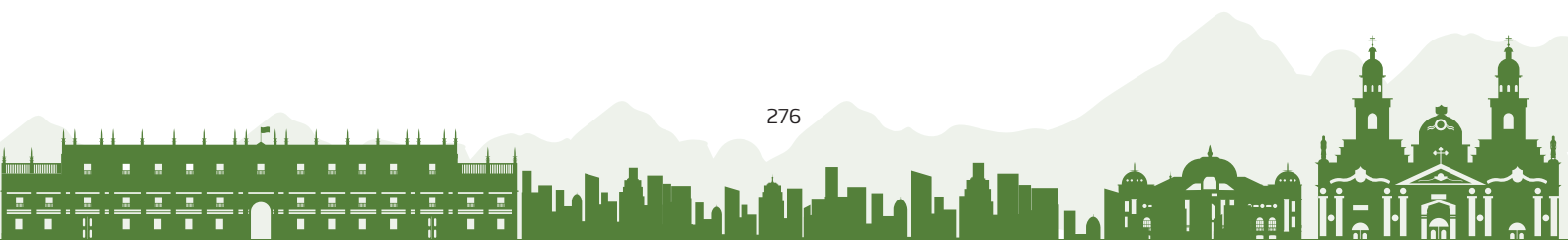
### PP-302 Architecture of a decision support system for future beehives

**Aleksejs Zacepins**<sup>1</sup>, Vitalijs Komasilovs<sup>1</sup>, Armands Kviesis<sup>1</sup>, Rob Mills<sup>2</sup>, Francesco Mondada<sup>2</sup>

<sup>1</sup>Latvia University of Life Sciences and Technologies, Faculty of Information Technologies

<sup>2</sup>Mobile Robotic Systems group, École polytechnique fédérale de Lausanne (EPFL), Lausanne, Switzerland

Honeybees are a key element in the food supply of humans on a global scale, as honeybees play a significant role as pollinators of various agricultural crops. With the development of information and communication technologies, internet of things, and data analysis methods, apiaries can be managed in more effective and modern ways. Individual beekeeper knowledge could be augmented by additional information sources. With the introduction of precision beekeeping which includes remote monitoring of honeybee colonies, beekeeping is already shifting from a traditional to modern and technological manner. The interdisciplinary Horizon 2020 project HIVEOPOLIS aims to develop a new approach in beekeeping, by creating novel beehive types, various novel mechatronic systems and proposing a new hive data infrastructure model. These intelligent HIVEOPOLIS beehives aim to help honeybees to cope with adverse environmental factors and increase the survival rate of the bee colonies themselves. This work presents a software architecture design for futuristic hives, providing infrastructure for efficient data management and ensuring operation of the decision support system. The decision support system developed upon this infrastructure enables closed-loop automated decision making. In-hive robotics systems, colony state identification, increasing of the number of available information sources, and advanced decision support have the potential to help and increase the resilience of honeybees that are essential to our nutritional supply.



### PP-303

## Multispectral microscopy imaging to detect purity of endemic Guindo Santo and Quillay honey using machine-learning tools against syrup adulteration

Guillermo Machuca<sup>1</sup>, Juan Pablo Staforelli<sup>2</sup>, Mauricio Rondanelli Reyes<sup>3</sup>, René Garces<sup>4</sup>, Braulio Contreras-Trigo<sup>1</sup>, Jorge Tapia<sup>5</sup>, Ignacio Sanhueza<sup>1</sup>, Anselmo Jara<sup>1</sup>, Iván Lamas<sup>3</sup>, Jose Troncoso<sup>6</sup>, Pablo Coelho<sup>1</sup>

<sup>1</sup>Facultad de Ingeniería, Arquitectura y Diseño, Universidad San Sebastián, Concepción 4080871, Chile.

<sup>2</sup>Departamento de Física, Universidad de Concepción, Casilla 160-C, Concepción 3349001, Chile.

<sup>3</sup>Laboratorio de Palinología y Ecología Vegetal, Departamento de Ciencia y Tecnología Vegetal, Universidad de Concepción, Campus Los Angeles, Juan Antonio Coloma 0201, Los Angeles 4451032, Chile.

<sup>4</sup>Facultad de Ciencias de la Naturaleza, Universidad San Sebastián, Concepción 4080871, Chile.

<sup>5</sup>School of Electrical Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso 2362804, Chile.

<sup>6</sup>Research Center in Natural and Exact Sciences, School of Education and Social Sciences, Adventist University of Chile, Chillan 3780000, Chile.

The global problem of food fraud is becoming a threat for the entire industry chain, from producers to consumers. In this work, we implement a system to detect the purity of Quillay (*Q. Quillaja saponaria*) and Guindo Santo (*GS. Eucryphia glutinosa*), which are two representative monofloral endemic honey from Chile. Both exclusive honeys suffer from syrup addition fraud, which increases the uncertainty of purity and limits their commercial growing.

Transmission hyperspectral microscopy and machine learning are used to obtain and analyze the spectral signature of not only pure Q honey, but also, for the first time, the GS honey. The information was used to determine whether the honey was adulterated with additional sugars or was pure by using classification algorithms based on artificial learning. The combination of both mentioned techniques allows to reduce the costs of a honey fraud detection equipment and time reduction for the analysis.

Using honey artificially adulterated with syrups at concentrations undetectable at naked eye, we reach an average accuracy value of 95.3% with using Linear discriminant analysis (LDA), Support vector machine (SVM), and Neural Network (NN).

### PP-304

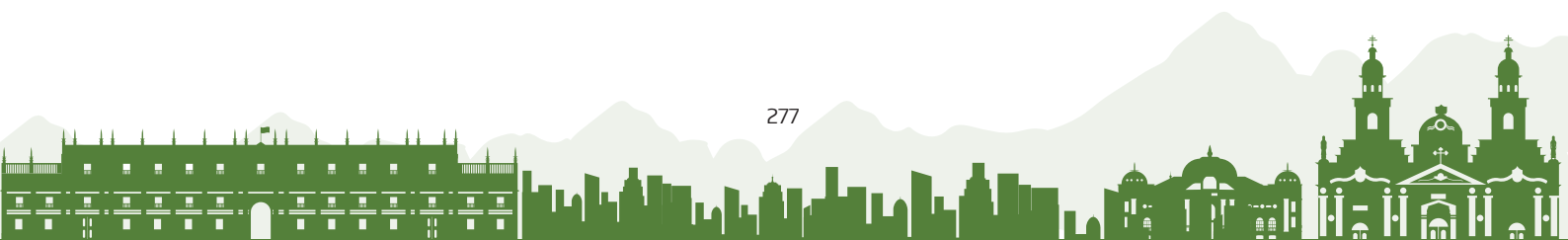
## Certification of botanical origin of "*Apis mellifera*" L. honey based on pollen analysis from some important honey-producing mesoregions in São Paulo State, Brazil

Cynthia Fernandes Pinto Da Luz<sup>1</sup>, Natalia Sêneda Martarello<sup>1</sup>, Paula De Souza São Thiago Calaça<sup>2</sup>

<sup>1</sup>Laboratório de Palinologia PALINO-IPA, Instituto de Pesquisas Ambientais, São Paulo, Brazil

<sup>2</sup>Serviço de Recursos Vegetais e Opoterápicos, Fundação Ezequiel Dias, Minas Gerais, Brazil

The botanical and geographical origin of the honey, as well as the quality control, are carried out by Melissopalynology, which analyzes pollen and other microscopic elements in the honey. However, botanical origin of honey by laboratory certification is not required by Brazilian legislation. Brazil is an important honey exporter. Driven by the favorable international scenario of increased demand for honey, São Paulo (SP) is the fourth Brazilian State in honey production. Nevertheless, most of the honey is marketed without certification of its botanical origin on the label. This study aimed to analyze 119 honey samples from SP by Melissopalynology, produced between 2007 and 2022, obtained in apiaries or on the market. The samples represent 11 of the 15 mesoregions of SP. Samples were processed without acetolysis. Pollen identification and quantification was based on the authors' expertise and consulting the Palynothesca PALINO-IPA of Instituto de Pesquisas Ambientais. A Principal Component Analysis (PCA) was performed on the relative frequency of each pollen type in the samples. We identified 185 pollen types, from 78 botanical families and 173 genera, Fabaceae being the richest, with 31 types. The PCA explained 27,74% of the data variability. The first component explained 18,22% and separated two groups of samples: one with high frequency of Citrus and other of Eucalyptus, showing the wide geographic occurrence of orange orchards and eucalyptus plantations in SP. The second component of PCA separated samples from the Mesoregion of Litoral Sul Paulista, Itapetininga and Macro Metropolitana Paulista/Microrregião Piedade with high similarity of pollen spectra due to the presence of native species of the Atlantic Rain Forest: *Croton*, *Euterpe*, *Holocalyx*, *Machaerium*, *Myrcia*, *Schinus*, *Struthanthus* and several *Asteraceae* species. The other group of samples was formed by samples of the Mesoregions of Bauru, Campinas, Macro Metropolitana Paulista, Metropolitana of São Paulo, Ribeirão Preto and São José do Rio Preto with high relative frequencies of pollen from *Althernanthera*, *Anadenanthera*, *Bidens*, *Mimosa bimucronata*, *Mimosa pudica*/M. *scabrella*, *Myrcia*, *Paullinia*, *Poaceae* and *Schizolobium*. The certification of botanical origin of honeys of São Paulo is important for their characterization and enables greater valuation of this product in the market.



### PP-305

#### Evaluation of a pilot site with 4 Langstroth hive management systems, through climate incidence in Cutaiqui, in the productive management 2019 - 2020

Guido Ernesto Saldias Urzagaste

Área Apícola, ONG NATIVA, Tarija, Bolivia

In the Great American Chaco, the climatic reality makes beekeepers increasingly concerned, since bee production depends on the climate, which has been highly variable in recent years. Through the Trinational Project "PROADAPT", the "Evaluation of a pilot site with 4 Langstroth hive management systems was carried out, through the climatic incidence in Caique, in the productive management 2019 - 2020". The Systems were: Single Chamber (SCS), Chamber and a Half (SCH), Double Chamber (SDC) and Superimposed Farrar (SSF), made up of 10 hives each, in 4 apiaries spaced 250 m apart., under similar conditions of vegetation, semi-shade, availability of water and the queen bees of the 40 hives were daughters of the same queen bee of the community, in the Livestock Property "Los Troncos" of the Cutaiqui Community of the Villa Montes Municipality. Information Recording Protocols were used on: Precipitation - Temperature - Flowering, State of the hives, Health (Varroosis), Production of nuclei, honey and economic analysis. With compliance with the protocols for one year, the following results were achieved. The analysis of precipitation (592.5 mm.) and minimum and maximum temperatures (0.5 and 44.5°C.), modulated the direct influence on the flowering curve. Categorization I, II and III, had 70%, 25% and 5% of the hives respectively. The infestation rate (by Varroa sp. from 3 monitoring) ranged from 0.7% (SDC) to 2% (SCS). 20 cores (most of the SDC) were produced. The yield of honey/Kg. /hive/system/year of the SCS was 65.9; SCH: 59.3; SDC: 44.1 and SSF 37.7. The economic evaluation established that the SFS required a greater economic investment (27.3%). The SCS achieved the highest production, honey yield and net balance in cash flow (10,722 Bs.), followed by the SCH, SDC and SSF. The other systems are not called "inefficient", because the results are consistent with the protocols executed in said environment and climate. Likewise, field journeys and socialization of results on Climate - Flowering - Beehive - Production (CFBP) were shared among beekeepers from the trinational Chaco with a resilient approach.

### PP-306

#### Evaluation of the chemical composition of Chilean honey using MALDI-TOF-MS technique and evaluation of their biological properties

Verónica Rachel Olate Olave<sup>1</sup>, Luis Guzmán Jofré<sup>2</sup>, Xaviera A. López Cortés<sup>3</sup>, Rafael Cornejo<sup>4</sup>, Fabiane M. Nachtigall<sup>5</sup>, Marnix Doorn<sup>4</sup>, Leonardo Silva Santos<sup>6</sup>, Arturo Bejarano<sup>4</sup>

<sup>1</sup>Instituto de Investigación Interdisciplinaria (I3), Universidad de Talca, Talca, Chile

<sup>2</sup>Departamento de Bioquímica Clínica e Inmunohematología, Facultad de Ciencias de la Salud, Universidad de Talca, Talca, Chile

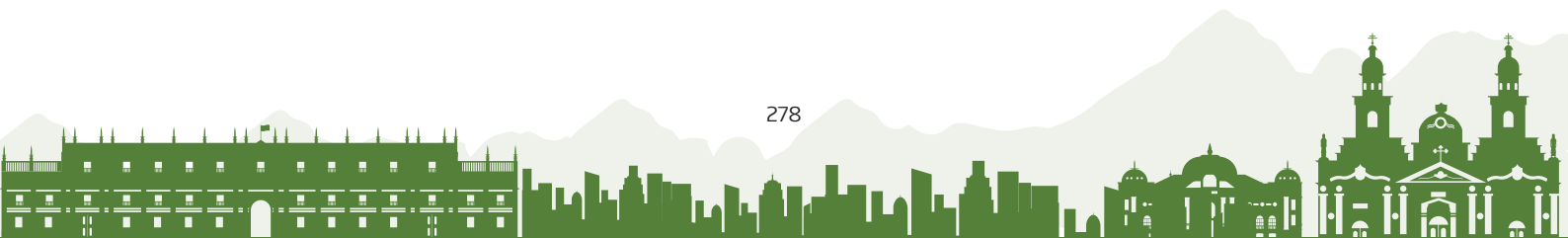
<sup>3</sup>Department of Computer Science and Industries, Universidad Católica del Maule, Talca, Chile

<sup>4</sup>Fraunhofer Chile Research, Santiago, Chile

<sup>5</sup>Instituto de Ciencias Químicas Aplicadas, Universidad Autónoma de Chile, Talca, Chile

<sup>6</sup>Laboratorio de Síntesis Asimétrica, Instituto de Química de Recursos Naturales, Universidad de Talca, Talca, Chile

Honey is the most famous natural sweet product with a high nutritional value. It contains numerous functional compounds, from which health benefits are obtained. In Chile, the production of honey is associated with its unique biodiversity, but it is exported mostly, as bulk honey. To increase the knowledge about the value of Chilean honey in terms of their chemical characteristics and biological activity, this work aimed to characterize the chemical and biological properties of 802 Chilean polyfloral honey samples from different geographical origins. The use of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) technique, combined with multivariate statistical analysis was introduced to study the chemical profiles. Using a complementary mass spectrometry method (GC-MS) 25 different constituents were identified in the studied honey, including hydrocarbons, acids, esters, glycoside isoprenoids, ketones, and a dihydroxyflavanone. The evaluation of biological properties in Chilean honey was measured in a representative number of polyfloral samples. For this purpose, the total phenolics and flavonoid content were measured in the selected samples. The antioxidant activity of the honey was evaluated through the ferric reducing antioxidant power assay (FRAP) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. On the other hand, the antibacterial activity was assessed by the dilution technique to determine the minimum inhibitory concentration (MIC) of the honey against seven bacterial strains, including Gram-positive and Gram-negative bacteria. The present work showed that the MALDI-TOF-MS technique combined with multivariate statistical analysis was useful to compare a large number of honey samples through the study of their chloroform extract profiles. However, the obtained profiles resulted in a complex mixture and this technique was not able to give the identity of the chemical markers. Regarding the biological properties, it was possible to characterize Chilean honey samples according to their antioxidant and antibacterial activity, which were highly variable. According to the statistical analysis, significant differences and some significant correlations were found in the biological activity of Chilean honey according to the year, origin, and/or chemical profiles. It means that, through the honey chemical profiles, it is possible to obtain information about its origin or its potential biological activity.



### PP-307

## Natural capsules of bee pollen as tailor-made controlled delivery systems for food applications

Seymanur Ertosun<sup>1</sup>, Volkan Aylanc<sup>1</sup>, Soraia I. Falcão<sup>2</sup>, Cristina Freire<sup>3</sup>, Miguel Vilas-Boas<sup>2</sup>

<sup>1</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; REQUIMTE-LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007, Porto, Portugal

<sup>2</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>3</sup>REQUIMTE-LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007, Porto, Portugal

Microencapsulation technology has become very popular in recent years and has been a subject of intense research. Microcapsules are applied in many different areas, from pharmacy, textiles, bioremediation and food technologies. These structures can be chemically produced with chitosan, cellulose, alginate, however they also may be obtained from capsules available in the nature. Plant pollens are one of the strongest biomaterials present in nature which can be easily found, and resistant to harsh chemical and physical conditions. Naturally they have different shapes and if empty, they can be used as microcarrier systems. In this work we described the preparation of natural capsules from different pollens types: *Rubus* spp., *Olea* spp. and *Erica* spp. Once collected, the bee pollen was separated by color and identified by palynological analyses. After confirming the purity, a two steps cleaning method was applied to ensure the elimination of the cytoplasmic content and also the proteinous structures which are responsible for most allergic reaction. The protein analysis of the raw pollen and clean natural capsules revealed, for *Rubus* spp., a decrease from  $23.7 \pm 0.1$  to  $4.4 \pm 0.0$  (g/100g), a trend observed in all pollen samples. The empty pollens were checked by scanning electron microscopy (SEM) to evaluate the main structure and its resiliency through harsh conditions. After, confocal laser scanning microscopy (CLSM) was used to certify the quality of the hollow capsules and to see the detailed structure of the samples. CLSM scanning showed that pollen samples were emptied without the cytoplasmic content. The behavior of these type of bee pollen gave promising perspectives for the application of the obtain microcapsules in different areas such as food technology. Their use to deliver specific substances such as food preservatives or additives will be the next stage under this project.

#### Acknowledgements

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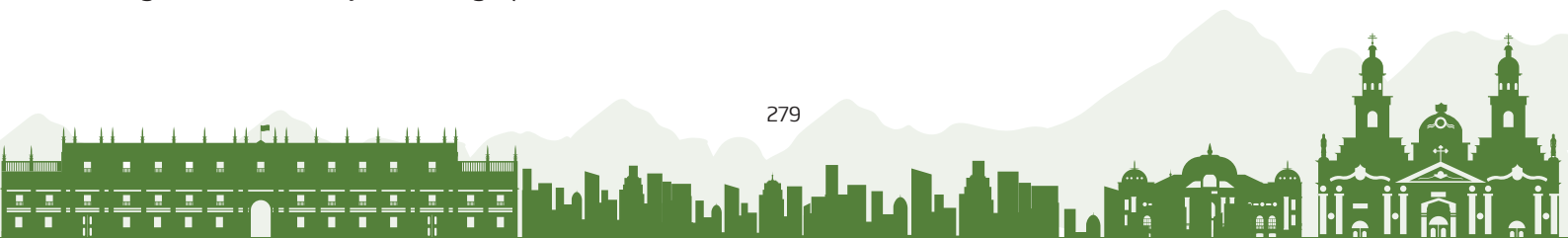
### PP-308

## Climate Smart Apiculture

#### Emily Bondor

Emily Bondor, Department of Horticulture, Cabrillo College Extension, Santa Cruz, California

Keeping bees is not easy, and the extreme weather events brought on by climate change are making it more difficult every year. From drought to deluge, heat waves to cold snaps, the bees' systems are stressed. The additive environmental stressors of climate change can then lead to an acceleration in colony failure on already weakened or compromised hives. The aim of this talk is to lay out some actionable and achievable steps towards keeping colonies in stasis through the increasingly unpredictable seasonal spikes of our modern climate. We as beekeepers need to stay one step ahead of the weather to help mitigate some of the harsh conditions that the climate crisis is creating. By utilizing both low-tech and high-tech management tools and techniques, we can help to curb losses that may otherwise occur due to extreme weather events. While this talk is being given by a beekeeper out of central California (USA), care will be taken to include considerations for the multiple different climate zones that encompass the apiaries of the global community attending Apimondia.





### PP-309

## Physicochemical quality control of honey produced in the Southern Region of Brazil

Ana Carolina Teixeira Ferreira, Tainá Simonetti, Ângela Junges, Liris Kindlein, Aroni Sattler

Department of Preventive Veterinary Medicine, Federal University of Rio Grande do Sul, Rio Grande do Sul, Brazil.

The control and monitoring of the honey quality guarantees to consumers the preservation of the product original characteristics and safety. Therefore, it is important to ensure that the physicochemical characteristics of the honey to be marketed comply with the legislation (Normative Instruction No. 11, October 20, 2000, MAPA). Among the main parameters analyzed in the routine quality control of honey; pH, acidity, moisture and hydroxymethylfurfural (HMF) values stand out. This study analyzed honey samples produced in honey/bee products extraction and processing warehouses in Rio Grande do Sul (Brazil) in order to identify the product compliance with identity and quality legal standards. The pH and acidity analyzes of 174 samples were determined using a potentiometer (ABNT NBR 15714-6/2020) and its moisture content using a refractometer (ABNT NBR 15714-2/2020). The HMF index was established by spectrophotometry (ABNT NBR 15714-9/2020) in 182 samples. Honey samples exhibited average values of: 4.16 for pH, 18.19 % for moisture content and 25.20 mEq.kg<sup>-1</sup> for free acidity. Acidity values directly influences the flavor of honey and its maximum limit is established at 50 mEq.kg<sup>-1</sup>(Codex Alimentarius, 2019). The maximum honey moisture content is set at 20% (IN N°11, 2000), since higher levels may indicate an inadequate harvest (Pita-Calvo, 2017). Despite not being required by Brazilian legislation, pH determination is an important indicator of honey quality, as it can influence the HMF speed formation which is originated from fructose dehydration processes in an acidic medium. The upper limit of HMF accepted by legislation is 60mg/kg and the mean value exhibited in the analyzed samples was 9.39mg/kg, although three samples (1.65%) showed values higher than those allowed (66, 72 and 152 mg/kg) therefore being considered unfit for consumption, since high levels may indicate prolonged storage, overheating or the addition of inverted sugars (Kukurova et. al, 2006; Brugnerotto et. al, 2021). Regarding the presented aspects, physicochemical analyzes are fundamental to guarantee the quality and identity of the honey, in addition to monitoring possible frauds, tampering or improper handling and ensuring the consumer a product in compliance with the legislation.

### PP-310

## Effect of a liquid diet with protein, vitamins and probiotic on physical-chemical and sensory quality of honey in *Apis mellifera iberica* and *A. mellifera ligustica* honey bees

Pedro Díaz Molins<sup>1</sup>, Antonio Martínez Mateo<sup>1</sup>, Raffaele Dall'olio<sup>2</sup>, José Serrano Marino<sup>3</sup>

<sup>1</sup>Zukán, S.L.

<sup>2</sup>Beesource

<sup>3</sup>University of Murcia

The aim of this study was to evaluate the influence of feeding honey bees with a new liquid containing 2% of protein, vitamins and probiotics, on the quality parameters of honey.

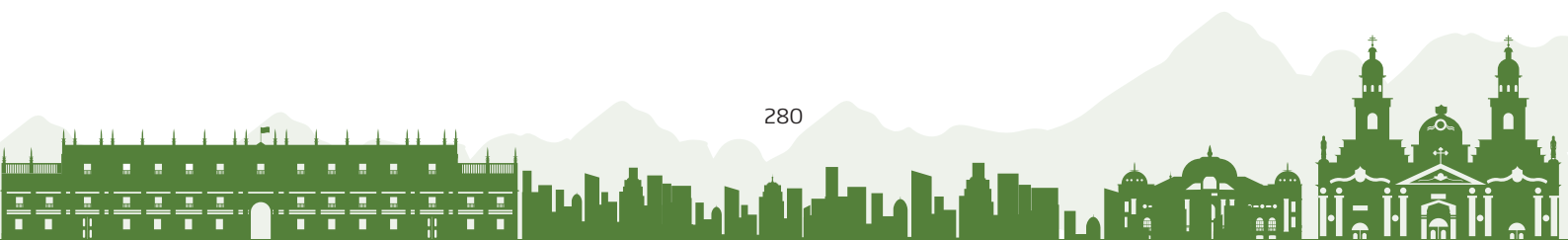
The experimental study was carried out in two locations, Murcia (Spain) and Bologna (Italy) from October 2021 to August 2022. Average temperature ranged between 12-26 °C in Murcia and 13-25 °C in Bologna. Ten hives were randomly placed in each location, of which five were a control group without feeding and the other five hives made up the feeding group. These were fed each two weeks with enzymatic inverted sugar syrup (75% d.s.), 2 % of protein (hydrolyzed yeast), vitamins (B1, B2, B3, B4, B5, B6, C and K) and probiotics (*Bacillus subtilis* and *Enterococcus faecium*). Starting date of feeding was 8th October 2021 (Bologna) or 5th November 2021 (Murcia). In total, Murcia hives were fed 11 times and Bologna hives 9 times.

Once the honeys were extracted, the quality parameters were analyzed according to the standards established in the European Honey Directive. We determined the following parameters: sugar profile, hydroxymethylfurfural, diastatic activity, °Brix, color, conductivity, pH, ashes and free acidity. The sensory quality (16 attributes) was analyzed by a trained panel (10 assessors) using an unstructured scale (10 cm).

Significant differences ( $p < 0.05$ ) were detected in °Brix, color, conductivity, diastatic activity and glucose content between control and feeding groups in both locations. However, values of these parameters are within the admitted range for human consumption by the EU Directive.

The sensory analysis showed that properties of the honey were similar in both groups although significant differences ( $p < 0.05$ ) were detected in some attributes as color, turbidity, odor and flavor. Again, the values were within the admitted range for commercial honey.

It is concluded that it is adequate to feed the hives with the liquid diet with protein, vitamins and probiotics, as it rises honey production (see complementary poster to this communication) and it doesn't affect the overall quality of the honey.





### PP-311

## Effect of a liquid diet with protein, vitamins and probiotic on honey production and winter survival in *Apis mellifera iberica* and *A. mellifera ligustica* honey bees

Pedro Díaz Molins<sup>1</sup>, Antonio Martínez Mateo<sup>1</sup>, Raffaele Dall'olio<sup>2</sup>, José Serrano Marino<sup>3</sup>

<sup>1</sup>Zukán, S.L.

<sup>2</sup>Beesource

<sup>3</sup>University of Murcia

The aim of this study was to evaluate the effect of a new liquid feeding with 2% of protein, vitamins and probiotics on the weight of hives and honey production, in managed Iberian and Italian honey bees.

The experimental study was carried out in two locations, Murcia (Spain) and Bologna (Italy) from October 2021 to August 2022. Average temperature ranged between 12-26 °C in Murcia and 13-25 °C in Bologna. Ten hives were randomly placed in each location, of which five were a control group without feeding and the other five hives made up the feeding group. These were fed each two weeks with enzymatic inverted sugar syrup (75% d.s.), 2 % of protein (hydrolyzed yeast), vitamins (B1, B2, B3, B4, B5, B6, C and K) and probiotics (*Bacillus subtilis* and *Enterococcus faecium*). Starting date of feeding was 8th October 2021 (Bologna) or 5th November 2021 (Murcia). All hives were weighed before and after feeding and honey of each group was weighed after extraction. In total, Murcia hives were fed 11 times and Bologna hives 9 times.

Consumption of food was higher in Murcia (8.4 kg/hive) than in Bologna (6.2 kg/hive), possibly because flowering occurred later in Murcia and thus the feeding time was extended. At the end of the experience the weight of the feeding group increased 2.61 kg in Murcia and 0.68 kg in Bologna. These results are in contrast to the weight decrease in the control group of Murcia, 3.55 kg, and in that of Bologna, 0.5 kg. Honey production of the feeding group was higher in Bologna (26.4 kg) than in Murcia (17.4 Kg honey/hive) although the proportional increase was higher in this last apiary (235% versus 64%).

It is concluded that the liquid diet with protein, vitamins and probiotics is effective to increase the production of honey and to assure a better hive survival during winter, when natural resources are almost vanished and weather conditions are hardly favorable.

### PP-312

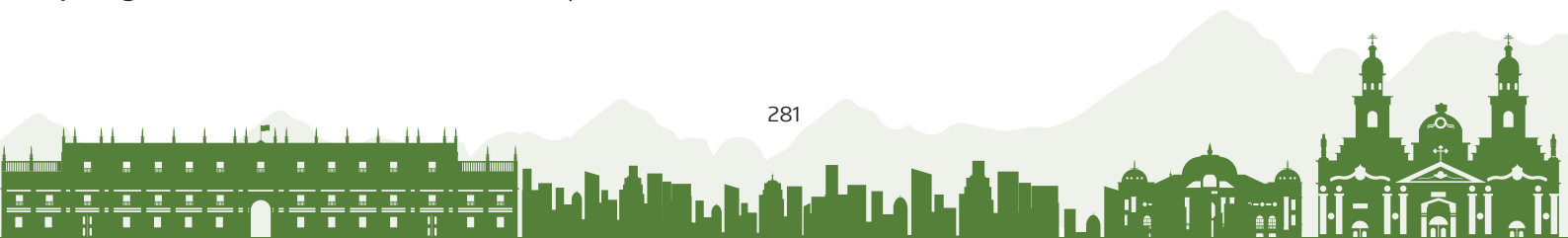
## Apitoxin from young bees has a higher concentration of mellitin

Valentina Micaela Rodríguez-batista<sup>1</sup>, Agustín Pérez<sup>2</sup>, José Anzola<sup>1</sup>, Catherine Fagúndez<sup>1</sup>, Enrique Nogueira<sup>1</sup>

<sup>1</sup>Facultad de Veterinaria, Universidad de la República, Uruguay

<sup>2</sup>Facultad de Agronomía, Universidad de la República, Uruguay

Apitoxin is made up of tens of bioactive substances synthesized by the bee that are deposited in the venom sac during the first 3 weeks of their adult life. Mellitin is the major component of the apitoxin, making up 40 to 50% of its dry weight, although some works report values higher than 70%. The traps used to harvest apitoxin are installed in different places in the hive that can be accessed by young or mature bees, which arises the question on whether different ages can determine different compositions on the apitoxin. The objective was to quantify the % of mellitin in the apitoxin of young and mature bees from the same hive. 6 hives of similar development (40,000 to 45,000 adult bees) were used, which were identified and later moved approximately 100 meters. In their place hives were left without adult bees that only contained combs with brood and food and empty combs. These hives were populated by the adult bees that occupied the transferred hives, since these have a fixed the place. This way by the end of the day the population of each hive was divided into 2 colonies, one with young bees and the other with mature bees. At dusk, the apitoxin extraction was carried out using ceiling traps for 30 minutes. The venom harvested from each hive was weighed and stored in a marked container, and then the % mellitin was quantified by HPLC. An analysis of variance was done to the values obtained, blocking by hive, with significant differences in the concentration of mellitin ( $P < 0.05$ ) of the apitoxin of young (64.81%) and mature (55.81%) bees. This may be because the components of the apitoxin are synthesized at different times and/or rhythms, so the time and place in the hive where the traps are installed to collect apitoxin must be considered, since in the same hive different compositions can be obtained according to whether young and/or mature bees access the traps.



### PP-313 Analysis of Bees Wax in "BEESEN-Honeycomb"

Jeong Dong Han, Jin Kyu Park  
BEESENBIO Co., Ltd

In the production of honeycomb honey in the off-season, differentiated technology that does not use standardized assembled bees wax-coated foundation can be used to produce high-quality specific bee's wax along with raw materials for pure honey sugar.

It was intended to suggest that the bees wax component of Beesen Bio's honeycomb is composed of pure natural wax only. Beeswax of natural hives contains 12-16% straight saturated hydrocarbons in the range of C27 to C33, and the higher the number of hydrocarbons with 33 or more carbon atoms are the lower the quality of natural Bee wax (Paul M., etc.). In order to verify whether artificial paraffin wax such as foundation is mixed or not in the BEESEN specification honeycomb, the Bees wax, wax of comb honey, and paraffin wax standard materials of the sample were analysed by GC, respectively. Paraffin wax is composed of hydrocarbons with 20 to 40 carbon atoms (Wikipedia). As a result, hydrocarbons were detected evenly from 7 to 12 minutes in both the paraffin wax standard materials and the grass, and the grass had a higher content ratio of C32 or higher after 10 minutes than the paraffin wax. Contrary to these analysis results, the beeswax of the Beesen honeycomb sample differed in all aspects, such as retention time, number of components, and content of the detection component compared to the GC chromatogram of the general honeycomb, and C24,28,32,36 detected as the main peak in the paraffin wax were not detected at all, so no evidence of partial incorporation was found. The GC analysis chromatograms of natural Beeswax (refined) and Beesen honeycomb Beeswax were completely consistent with the retention time and composition patterns of major peaks from 8 to 11 minutes. In addition, the Bees wax of the BEESEN honeycomb did not detect two hydrocarbons of more than 12.74 and 14.36 minutes C33 from the natural Bees wax standard. This shows that the BEES WAX of the BEESEN honeycomb sample is a high-quality natural BEESWAX with fewer high-carbon hydrocarbons with 33 or more carbon atoms.

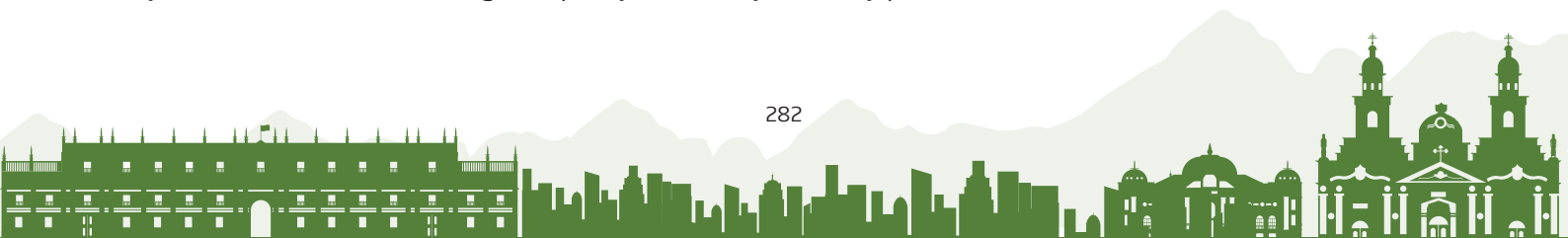
### PP-314 Profiling Botanical Origin and Assessing Quality of Honey from Fujairah, United Arab Emirates as an Implications for Local Production and Consumer Protection

James Arruda Salome, Shaher Bano Mirza, Fouad Lamghari Ridouane, Khawla Mohammed  
Alyammahi  
Fujairah Research Centre

This study aimed to profile the botanical origin elements of Fujairah's honey and assess the significance of botanical origin identification practices on honey quality. The Emirate of Fujairah, located in the United Arab Emirates, is known for its diverse bioclimatic areas, providing optimal conditions for apiculture activities. Honey produced by local honeybees is considered an essential element of the national food basket due to its nutritional value and benefits. Therefore, assessing Fujairah's honey's botanical origin and quality is crucial for consumer protection and for promoting local production.

Physiological analysis and pollen identification tests were conducted on honey samples collected from different bioclimatic areas, apiary locations, and honey production seasons to achieve the study's objectives. The study aimed to determine the type of origin forage plants, blooming stages, and their correlation with climatic factors. The results showed that the botanical origin of Fujairah's honey is diverse, with more than 28 plant species identified from the pollen analysis. The honey samples were classified as monofloral or polyfloral based on the type and quantity of pollen present. The study found a significant correlation between honey quality and the bioclimatic conditions and forage plants visited by honeybees.

The long-term goal of this study is to create and standardize the quality of Fujairah's honey by defining the adulteration level in the UAE honey market and assessing adulterant identification methodologies using advanced techniques. The findings of this study contribute to the development of honey standards and the promotion of local honey production at a fundamental level. By identifying the botanical origin of honey, consumers can ensure the authenticity and quality of the product they are purchasing. Moreover, the findings have implications for the honey industry and contribute to ensuring the quality and safety of honey products.



### PP-315 Heavy metal content in bee products from urban and rural apiaries

Zbigniew Koltowski<sup>1</sup>, Wioletta Popińska<sup>2</sup>

<sup>1</sup>The National Institute of Horticultural Research, Apiculture Division, Pulawy, Poland

<sup>2</sup>The National Institute of Horticultural Research, Horticulture Products Quality Laboratory, Skierniewice, Poland

One of the goals of the conducted research is to assess the quality of bee products obtained from agricultural areas and urban apiaries. In the current report, we present the results of heavy metal content in honey and bee bread samples from various locations. The samples of bee products obtained from apiaries stationed in urban agglomerations (Warsaw, Lublin, Kraków, Upper Silesia and Puławy) and from apiaries in the vicinity of these places but located in agricultural areas were compared. The samples of honey (n=40) and bee bread (n=23) was collected in 2021-2022. The content of arsenic (As), cadmium (Cd) and lead (Pb) was determined by mass spectrometry with coupled plasma ionization (ICP-MS), while the content of mercury (Hg) was determined by atomic absorption spectrometry (ASA) using the amalgamation technique. It was found a much lower content of the tested heavy metals in the samples of honey than in the samples of bee bread. The honey samples from 2021 had an average higher content of only Cd and Pb than the samples from 2022. Comparing the examined locations, honey from the city had a slightly higher content of As and Hg, but lower content of Cd and Pb compared to honeys from rural areas. In the bee bread samples, a higher content of all tested heavy metals was found in 2022 compared to 2021. There was definitely more Pb in the bee bread from the city than from agricultural areas, but clearly less Cd and Hg. The maximum level of Pb for honey specified in Commission Regulation (EU) 2021/1317 of 9 August 2021 was not exceeded in individual honey samples, apart from the one sample from agricultural area. If these standards are applied to bee bread, most of the samples exceeds the limit, especially in the samples from 2022. One sample of bee bread from farmland was also of concern for Hg content, which was extremely higher from the levels found in other samples.

### PP-316 Honey certification pefc under sustainable forest management

Patricio Rojas<sup>1</sup>, Rodriigo Vidal<sup>2</sup>, Maria Paz Molina<sup>3</sup>, Francisco Rodriguez<sup>4</sup>, Jaime Espejo<sup>5</sup>, David Gutierrez<sup>6</sup>

<sup>1</sup>PEFC CHILE. rodrigo.vidal@pefc.cl

<sup>2</sup>INFOR, Sede Biobio. mmolina@infor.cl

<sup>3</sup>Forestal Mininco. francisco.rodriguez@cmpec.cl

<sup>4</sup>Consultor. jespejoc@uc.cl

<sup>5</sup>Forestal Arauco. David.Gutierrez@arauco.com

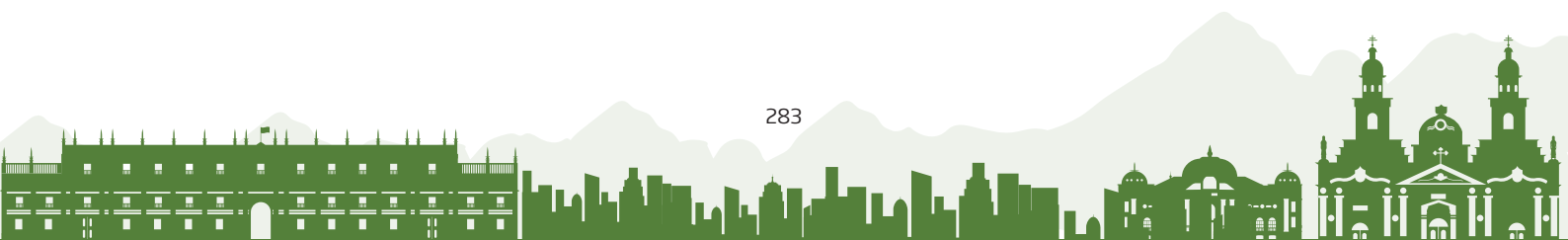
<sup>6</sup>INFOR. SEDE METROPOLITANA parojas@infor.cl

During the months of October 2019 and April 2020, a Pilot Project was developed with the objective of certifying the production of honeys under sustainable forest management PEFC Chile in the heritage of the companies Forestal Mininco and Forestal Arauco with beekeepers from the communes of Mulchén, Huepil, Cañete and Los Alamos. This with the additional objective of giving greater added value to the production of honeys for their commercialization in the local and international market of beekeepers in the VIII Region of Biobio and also incorporating the Eucalyptus spp plantations as food for apiaries.

Forest companies permit access facilities to beekeepers, to jointly defined properties, for the establishment of apiaries in sectors with forest plantations, nuclei of native forest and areas undergoing restoration, where bees not only fed and developed prosperously if not increased their honey production.

PEFC Chile undertook to coordinate the audit process between the Certification Body and Beekeepers under the Chain of Custody Standard and to produce certified honeys from sustainably managed forests, to train and advise beekeepers at the standard implementation stage; coordinate the audit process between the Certification Body and the beekeepers. It financed the Certification Body fees the first year of the audit and will exempt the annual fee for use of the PEFC Chile brands, for the 5-year cycle.

The beekeepers paid the Certification fees for the first year of the audit and subsidized the payment of the annual fee to the PEFC Chile Corporation for use of the PEFC brand or logo for a 5-year cycle. They also promised to pay the Certification Body fees from the second year. The beekeepers belong to the Biobio Region, preferably selected for being part of the National Beekeeping Network and with significant experience in beekeeping production and the Official Animal Traceability Program of the SAG. The beekeepers promised to comply with all the documentation associated with the implementation of the PEFC Chile Chain of Custody Standard and to participate in the audit process to certify their honey production in certified forests.



### PP-317 Phytochemical analysis of Saudi Arabia Moringa honey

Ahmed G. Hegazi<sup>1</sup>, Ahmed F.m. Al Gethami<sup>2</sup>, Faiz M. M. Al Guthami<sup>2</sup>, Mohamed Ramadan<sup>2</sup>

<sup>1</sup>NATIONAL RESEARCH CENTRE, DOKKI, GIZA, EGYPT

<sup>2</sup>AL GUTHAMI FOUNDATION, SAUDI ARABIA

**OBJECTIVE:** The aim of the study was to characterize varieties of Moringa honey (un floral and polyfloral) from Saudi Arabia based on physicochemical parameters (moisture content, pH, hydroxymethylfurfural, acidity, water activity, and diastase activity) and carbohydrate profile (glucose, fructose, total glucose-fructose, and glucose-fructose ratio). **MATERIAL-METHODS:** The fresh 166 honey samples (3 kg of each) were kindly provided by the Alnahal aljwal Company, 2021 flowering season. The honey sample was collected in a sterile universal glass container and kept at 2–8°C until tested. Phytochemical analysis was done. Determination of sediment content, total grains, moisture content, water-soluble solids, acidity, electrical conductivity, total sugars content, inverted sugars, glucose (g/100 g), fructose (g/100 g), total glucose + fructose, fructose/ glucose ratio, sucrose (g/100 g), diastase enzyme activity and HMF were calculated. **RESULTS:** A Phytochemical analysis of honey samples was varied. All the physicochemical parameters studied were significantly different ( $P < 0.05$ ) among all honey varieties. The results of the analysis were compared with Saudi National Standard, Codex standard, and published data in the literature. **CONCLUSION:** It was obvious that the honey quality was varied based on the botanical origins.

### PP-318 Physiochemical analysis of different Acacia honey types in Saudi Arabia

Ahmed G. Hegazi<sup>1</sup>, Faiz M Al Guthami<sup>2</sup>, Mohamed Ramadan<sup>2</sup>, Ahmed Al Gethami<sup>2</sup>

<sup>1</sup>NATIONAL RESEARCH CENTRE, DOKKI, GIZA, EGYPT

<sup>2</sup>AL GUTHAMI FOUNDATION, SAUDI ARABIA

**BACKGROUND:** Honey had been well documented as oldest traditional medicines. Its use is recorded in Egyptian papyri dated from 1900 to 1250 B.C. which used many of the Egyptian prescriptions including honey. Honey enhancing the growth of tissues for wound repair, suppress inflammation, and bring about rapid autolytic debridement. The objective of this study was aimed to determine the Physiochemical analysis of some Saudi Arabia honey. **MATERIAL-METHODS:** The fresh 66 honey samples (3 kg of each) were kindly provided by Alnahal aljwal Company, 2020 flowering season. Honey sample was collected in a sterile universal glass container and kept at 2– 8°C until tested. Physiochemical analysis was done. Determination of sediment content, total grains, moisture content, water soluble solids, pH, acidity, electrical conductivity, ash content, total protein content, sugars content, inverted sugars, glucose (g/100 g), fructose (g/100 g), fructose/ glucose sucrose (g/100 g) diastase enzyme activity, and HMF were performed. **RESULTS:** Physiochemical analysis of honey samples were varied according to different honey sources. It was obvious that the honey quality was varied based on the botanical origins.

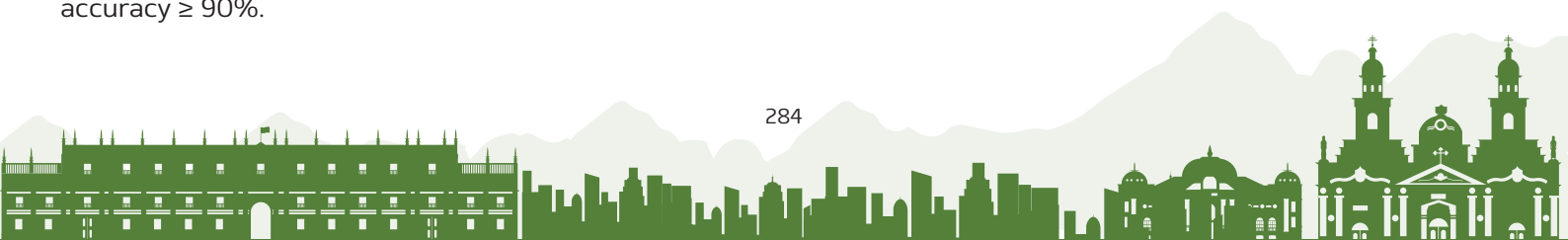
### PP-319 Authentication of bee products from the Atlantic Forest, Brazil

Fernanda Susi Luccas<sup>1</sup>, Elisabete A. De Nadai Fernandes<sup>1</sup>, Yuniel Tejada Mazola<sup>1</sup>, Márcio Arruda Bacchi<sup>1</sup>, Gabriel Adrián Sarriés<sup>2</sup>, Adriana De Angelis Fogaça<sup>1</sup>

<sup>1</sup>Nuclear Energy Center for Agriculture, University of São Paulo, Laboratory of Radioisotopes, Piracicaba, Brazil

<sup>2</sup>Luiz de Queiróz College of Agriculture, University of São Paulo, Engineering Department, Piracicaba, Brazil

Honey, propolis, waxes and bee bread are consumed worldwide due to their nutritional and pharmacological properties that boost human health. Safety is a matter of concern, as honey is the third most counterfeit product in the world. Demonstrating the authenticity of bee products becomes a prerogative to overcome trade barriers. Brazil is the 7th world exporter of honey recognized by its biodiversity, having more than 300 species of meliponine bees and 2 million hives of "Apis mellifera sp" concentrated mainly in the Atlantic Forest. Covering 17 different states along 3000 km of the Atlantic coast and occupying 15% of the Brazilian territory, the biome is home to 72% of the population and produces 50% of all food consumed in the country, mainly vegetables, intrinsically dependent on pollination by bees. Despite its historical degradation, the Atlantic Forest is currently protected by law, where its economic activities generate the lowest GHG emissions in the country, being an auspicious scenario for beekeeping and meliponiculture. In this sense, the chemical characterization of Atlantic Forest bee products can be a powerful tool to assess their authenticity. Therefore, a comprehensive sampling of honey, propolis, wax and bee bread from the meliponine bee "Tetragonisca angustula" (Jataí) and the hybrid honey bee "Apis mellifera sp" was performed in the biome. Advanced analytical techniques, such as neutron activation analysis (NAA) and triple quadrupole inductively coupled plasma mass spectrometry (TQ-ICP-MS), allowed determining 30 chemical elements (Ag, Al, As, Ba, Be, Br, Ca, Ce, Co, Cr, Cs, Cu, Fe, Ga, Gd, K, La, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Th, Ti, U, V and Zn). Applying artificial intelligence to the elemental mass fractions provided classification models to discriminate bee products, according to their entomological origin, with an accuracy  $\geq 90\%$ .





## Beekeeping Technology and Quality

### PP-320

#### Brood production and food reserve in colonies of Africanized bees *Apis mellifera* sp., created in a mangrove area with provision of alternative protein feeds

Eleuza Gomes Tenório, Euler Gomes Tenório, Helder Luis Chaves Dias, Jennyfer Sousa Araújo

Zootecnie Departament, State University Of Maranhão, Brazil

One of the alternatives for the sustainable use of mangroves would be the creation of bees to produce honey. Among beekeepers there is a knowledge that mangrove areas are excellent bee pastures in supplying nectar in abundance, leading to a high production of honey. However, one of the main difficulties pointed out by beekeepers who annually migrate their colonies to mangrove areas in Maranhão is the lack of pollen. The insufficiency of this food causes a weakening of the colonies, compromising the production of honey in the following harvest. In addition, malnutrition and the stress caused by the lack of food facilitate the emergence of diseases and the attack of natural enemies. The objective of this work was to evaluate the brood production of Africanized bees *A. mellifera* sp. with the provision of alternative protein foods to supply pollen deficiency, in colonies installed in a mangrove area in Campos de Perizes, municipality of Bacabeira, Maranhão, Brazil. Thirty colonies were evaluated using four formulations, with the following alternative foods: leucena leaf *Leucaena leucocephala* (F. Fabaceae), cassava leaf *Manihot esculenta* (F. Euphorbiaceae), "sabiá" leaf *Mimosa caesalpinifolia* (F. Fabaceae) and babassu cake *Attalea speciosa* (F. Arecaceae). These were compared with soybean meal *Glycine max* (F. Fabaceae). Feeding performance was measured by mapping the combs in the nests of experimental colonies. Brood cells (open and closed, worker and drone), pollen cells and honey cells were counted. The results showed that the treatments were like each other. Artificial feeding is an important management technique for beekeepers, especially migratory ones that bring their hives to the mangrove region. The supply of leucaena leaf flour, cassava, sabiá, in addition to flour from babassu cake could be used to replace soybean meal, in an environment poor in pollen resources. Furthermore, these foods are widely available and inexpensive.

### PP-321

#### Melissopalynological Characterization of the Locality of Malimán (Iglesia Department, Province of San Juan)

María Virginia Escobar Martínez<sup>1</sup>, María Susana Martínez<sup>2</sup>

<sup>1</sup>Instituto Nacional de la Agricultura Familiar, Campesina e Indígena, Jefatura de Gabinete de Ministros. Presidencia de la Nación Argentina

<sup>2</sup>Facultad de Ciencias Exactas, Físicas y Naturales. Universidad Nacional de San Juan. Argentina

Melissopalynology is the study that allows identifying the botanical origin of pollen in hive products to plan the quality and sustainability of them.

Due to its special microclimate, Iglesia department, situated in the northern part of San Juan province, has a great beekeeping development.

The locality of Malimán had a productive boost thanks to projects granted by the National Parks Administration, including one for Beekeeping Development, which we worked on. One of the goals of this project was to identify the beekeeping flora from which *Apis mellifera* L. collects nectar and forms pollen grains.

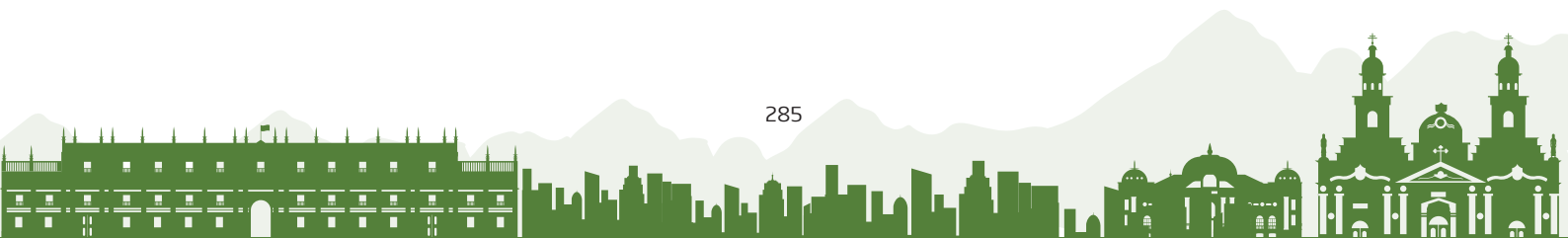
At the first stage, to characterize the beekeeping flora of Malimán, fieldwork was carried out with the participation of residents. Specimens of all plant species were collected to build a palynothèque of reference. Bee products were collected from three apiaries, and pollen grains from traps and samples of ripe honey harvested by producers were obtained.

The second stage was carried out at the Current and Quaternary Palynology Laboratory of the Mendoza Scientific and Technological Center, in Argentina (IANIGLA, CCT). The identification of pollen species in honey and pollen grains was performed by comparison with the palynothèque of reference.

According to the data obtained, the analyzed honeys are unigeneric or unifloral, exceptionally unispecific. In the Qualitative Analysis of Honeys, the highest percentages correspond to pollen from the *Prosopis* genus, *Tessaria absinthioides* standing out, too.

From the analyzed pollen grains, 82% belong to grains with native species pollen. The most abundant monogeneric and monospecific grains are those formed with pollen from *Prosopis* spp. and *Tessaria absinthioides*. The remaining 18% is pollen from cultivated species, highlighting the 9.14% corresponding to *Medicago sativa*.

The study demonstrated that *Apis mellifera* L. has a preference for native species, especially those in the *Prosopis* genus. Therefore, it is recommended that locals preserve and take care of their native flora, not only to reap its multiple benefits but also because it is an excellent resource for beekeeping in their apiaries.





### PP-322

#### Description of the sugar composition and antioxidant activity of honey samples obtained from "clean" areas of the Central-Southern region of Chile

Pablo A Ulloa Fuentes<sup>1</sup>, Mariela Silva<sup>2</sup>, Rafael Melo Freire<sup>1</sup>, Daniela Olivares<sup>1</sup>, Marcela Valenzuela<sup>1</sup>, Marcela Fuentes<sup>1</sup>, Ximena Faúndez<sup>1</sup>, Iris Lobos Ortega<sup>2</sup>

<sup>1</sup>Instituto de Investigaciones Agropecuarias (INIA), La Platina, Santiago, Chile

<sup>2</sup>Instituto de Investigaciones Agropecuarias (INIA), Remehue, Osorno, Chile

Honey is a complex mixture of different nutrients and components, the percentages of which vary according to a number of numerous factors. Most honeys share only about 80% of their physical and chemical composition. Differences in composition and functional activities can result from geographical and environmental conditions, the botanical source feed, the type of bee producing the honey, and the extraction method used. This generates different colors, viscosity, taste and properties. Therefore, needs to research honey from different regions in order to obtain its global properties. This is why it is necessary to know the composition, bioactive compounds (phenols and flavonoids), antioxidant activity and presence of residues of honey from the Central-Southern region of Chile. Honey samples (n=111) were collected in different zones (latitude 36°37'00" south and longitude 71°57'00" west to the north; up to latitude 43°40'56.64" south and longitude 72°0'25.42" west to the south). This sector has a great diversity of flora, made up of native species, endemic species and exotic species which contribute and differentiate the honey production. All samples were harvested March-April 2020 directly by the beekeepers. Samples were analyzed in sugars content (fructose, glucose and sucrose), total phenolic/flavonoid contents, antioxidant activity (DPPH free radical) and presence of residues. Monosaccharides results were lower than those normally found in honey. Fructose content showed range of 14.6-40.9%, glucose 10.2-35.8% and sucrose 0.6-2.8%. For the bioactive compounds, the honey samples showed a range from 5.43 to 17.8 (mg GAE/g honey) in relation to the total phenolic content. For the total flavonoid a range from 4.08 to 19.30 (mg QE/100 g honey). Antioxidant activity was at the level of 3.9 to 63.1%. In addition, the multiresidue analysis (LD = 10 ppb; LOQ = 5 ppb) of the samples showed the absence of pesticide residues. Therefore, taking into account all the results, it is fair to say that the honey collected in the area is a good source of bioactive compounds and functional activity, as well as being of good quality and without pesticide residues. This confirms that the zones are still free from agricultural interference due to the use of pesticides.

### PP-323

#### Valorization of Eucalyptus Honey from Uruguay: Physicochemical Characterization, Volatile Profile and Biological Activity

Lucía Almeida Kutscher<sup>1</sup>, Gloria Daners<sup>2</sup>, Jessica Báez<sup>3</sup>, Ana Acevedo<sup>4</sup>, Laura Fariña<sup>1</sup>

<sup>1</sup>Área Enología y Biotecnología de las Fermentaciones, Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Química, UdelaR, Montevideo, Uruguay.

<sup>2</sup>Departamento de Paleontología, Instituto de Ciencias Biológicas, Facultad de Ciencias, UdelaR, Montevideo, Uruguay.

<sup>3</sup>Laboratorio de Bioactividad y Nanotecnología de Alimentos, Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Química, UdelaR, Montevideo, Uruguay.

<sup>4</sup>Departamento de Bioquímica Clínica, Facultad de Química, UdelaR, Montevideo, Uruguay.

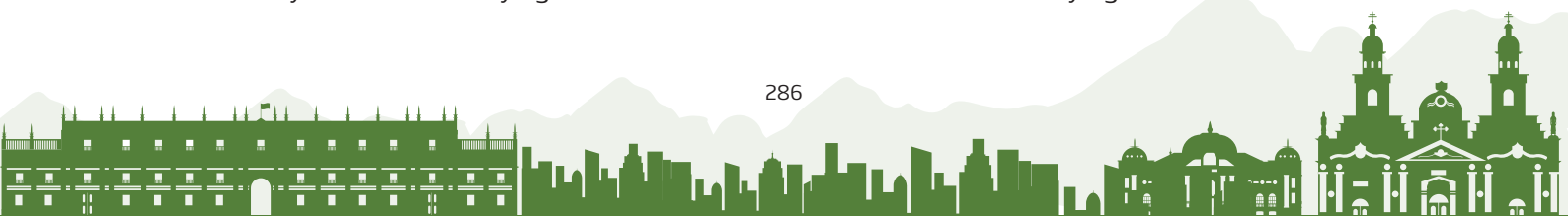
Eucalyptus plantations have been established in many areas of the world due to their fast growth and profitability. Uruguay has promoted a national policy for the development of the forestry sector that has resulted in more than 1 million hectares of plantations dominated by the Eucalyptus genus. Among the varieties with the largest planted area are Eucalyptus grandis, Eucalyptus globulus and Eucalyptus dunni.

The beekeeping sector has taken advantage of this situation to focus part of its production on honey from Eucalyptus forests, achieving a honey that is characteristic and pure honey in terms of its quality, production method and worldwide scarcity. This type of honey can more easily fall within the criteria of organic production and it has a specific market that demands for it.

This study aims to characterize Eucalyptus honey from Uruguay. In this context, 38 honeys from seven departments from Uruguay were analyzed during two consecutive harvests.

A pollen analysis and a battery of basic physicochemical analyses (pH, acidity, conductivity, humidity, color, hydroxymethylfurfural, diastase, ash and sugars) were performed. Volatile compounds were extracted by SPME and subsequently analyzed by GC-MS. Nutraceutical characteristics, such as total polyphenol content, flavonoid content and antioxidant capacity, were studied. In addition, a screening was carried out to determine antimicrobial activity against Staphylococcus aureus and Escherichia coli. The concentration of hydrogen peroxide was also determined in each of the samples.

The values obtained in the physicochemical analyses complied with the bromatological regulations. Reference values for color, sugar profile, ash and conductivity were obtained for this honey. The values obtained for polyphenols, flavonoids and antioxidant activity were compared with the values obtained for Uruguayan honeys and others recognized worldwide, such as Manuka honey. Volatile chemical markers already reported to be specific in Eucalyptus honey were detected. Also, new compounds specific to Eucalyptus honey from Uruguay were found. It was observed that 84.1% of honeys showed activity against S. aureus while 45.5% showed activity against E. coli.



### PP-324

#### Characterization as a Strategy to Create Value: Study of Sorghum Honeydew Honey in Uruguay

Lucía Almeida Kutscher<sup>1</sup>, Florencia Agustina Olivera<sup>1</sup>, Gloria Daners<sup>2</sup>, Eduardo Dellacassa<sup>3</sup>, Laura Fariña<sup>1</sup>

<sup>1</sup>Área Enología y Biotecnología de las Fermentaciones, Departamento de Ciencia y Tecnología de los Alimentos, Facultad de Química, UdelaR, Montevideo, Uruguay.

<sup>2</sup>Departamento de Paleontología, Instituto de Ciencias Biológicas, Facultad de Ciencias, UdelaR, Montevideo, Uruguay.

<sup>3</sup>Laboratorio de Biotecnología de Aromas, Departamento Química Orgánica, Facultad de Química, UdelaR, Montevideo, Uruguay.

One way to add value to honeys and other hive products is through their physicochemical characterization. Honeydew honey, unlike floral honeys, are hive products obtained from insect secretions with a high sugar content, instead of nectar. This makes them chemically and organoleptically different from commercial honeys.

Currently, due to the increase in monoculture, there are large extensions of sorghum plantations in Uruguay. These have an aphid that secretes this type of substances, causing hives adjacent to these crops to produce sorghum honeydew honey.

Although the production of this type of honey is not desired by beekeepers, in many cases, depending on variables unrelated to the them, large quantities of this product are obtained. The problem is that these honeydew honeys are often difficult to market and, in many cases, can hinder the exportation of floral honeys. These may appear to be adulterated if they are accidentally mixed with honeydew ones.

The aim of this study is to characterize these products and evaluate its nutraceutical properties. To achieve this, pH, acidity, conductivity, moisture, color, hydroxymethylfurfural, diastase and ash analyses were carried out in 15 samples of sorghum honeydew honey. At the same time, their nutraceutical properties, such as total polyphenol content and antioxidant capacity, were studied. Determination of hydrogen peroxide was also included in this study.

The values obtained in the physicochemical analyses complied with the bromatological regulations. Reference values for pH, acidity, color, moisture, ash and conductivity were obtained for these honeys. They are characterized for their high conductivity and amber color. The values obtained for nutraceutical activities were compared with the values obtained for Uruguayan honeys, honeydew honeys and others recognized worldwide, such as Manuka honey.

### PP-325

#### New green propolis extract presentations as promising ingredients that could substitute green propolis hydroalcoholic extract

Andresa Aparecida Berretta<sup>1</sup>, Luana Gonçalves Zamarrenho<sup>1</sup>, Luana Gonçalves Zamarrenho<sup>2</sup>, Juliana Arcadepani Correa<sup>1</sup>, Jéssica Aparecida Lima<sup>1</sup>, Giovanna Bonfante Borini<sup>1</sup>, Sergio Ricardo Ambrosio<sup>3</sup>, Hernane da Silva Barud<sup>4</sup>, Jairo Kenupp Bastos<sup>5</sup>, David De Jong<sup>6</sup>

<sup>1</sup>Research, Development & Innovation Department, Apis Flora Indl. Coml. Ltda., Ribeirão Preto, Brazil

<sup>2</sup>Ribeirão Preto School of Philosophy, Sciences and Letters, University of São Paulo (FFCLRP/USP), Ribeirão Preto, Brazil

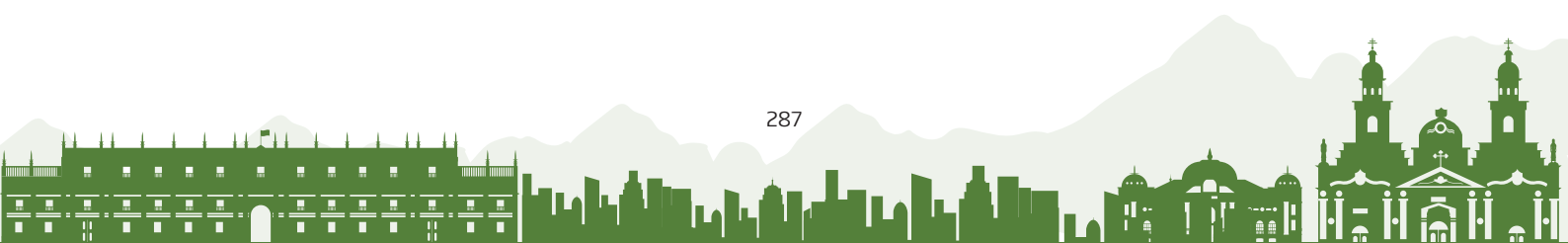
<sup>3</sup>University of Franca (UNIFRAN), Franca, Brazil

<sup>4</sup>University of Araraquara (UNIARA), Araraquara, Brazil

<sup>5</sup>School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (FCFRP/USP), Ribeirão Preto, Brazil

<sup>6</sup>Ribeirão Preto Medical School, University of São Paulo (FMRP/USP), Ribeirão Preto, Brazil

The technologies used to produce the different dosage forms of propolis can selectively affect the original propolis compounds and their biological activities. The most common type of propolis extract is hydroethanolic. However, there is considerable demand for ethanol-free propolis presentations, including stable powder forms. Three propolis extract formulations were developed and investigated for chemical composition, antioxidant and antimicrobial activity: polar propolis fraction (PPF), soluble propolis dry extract (PSDE), and microencapsulated propolis extract (MPE). The different technologies used to produce the extracts affected their physical appearance, chemical profile, and biological activity. PPF was found to contain mainly caffeic and p-coumaric acid, while PSDE and MPE showed a chemical fingerprint more close to the original green propolis hydroalcoholic extract used. MPE, a fine powder (40% propolis in gum Arabic), was readily dispersible in water, and had less intense flavour, taste, and color than PSDE. PSDE, a fine powder (80% propolis) in maltodextrin as a carrier, was perfectly water soluble and could be used in liquid formulations; it is transparent and has a strong bitter taste. PPF, a purified solid with large amounts of caffeic and p-coumaric acids, had the highest antioxidant and antimicrobial activity. All three had antioxidant and antimicrobial properties and could be used in products tailored to specific needs.



### PP-326

#### Pollen spectrum of honey from *Apis mellifera* sp. produced at Quinta, São José de Ribamar, Maranhão, Brazil

Eleuza Gomes Tenório, Maria daConceição Barbosa de sousa, Monique Ribeiro, Helder Luis Chaves Dias  
ZOOTECNIE DEPARTAMENT, STATE UNIVERSITY OF MARANHÃO, BRAZIL

Knowledge of the plants in a region, their flowering time and pollen characteristics, help in determining the plant species that contribute to the composition of honey, for the preservation of flowering and management of bees in natural, agricultural and urban environments. The objective of this study was to identify, through the pollen spectrum present in honey, the botanical species used by *Apis mellifera* sp. Honey samples were collected in the five-year harvests (2010, 2015, 2016, 2017 and 2019), when extractions were made from a set of 10 colonies. The acetolysis method was adopted for pollen identification. Up to 500 pollen grains were counted from each sample, establishing the percentages and classes of occurrence of each pollen type. The results showed 36 pollen types found in the honey samples, distributed in 17 families and 28 genera. The families with the highest representation in number of species were Fabaceae (nine species), Myrtaceae (four species), Rubiaceae (four species), Lamiaceae (three species), Amaranthaceae (two species), Asteraceae (two species) and Malvaceae (two species). The other families were found only one pollen type. Although there is a diverse vegetation in the study area, few pollen types were observed in the honey samples in 2010 (10 types), in 2015 (7 types) and in 2016 (10 types). On the other hand, in 2017, 19 pollen types were observed and, in 2019, 25 pollen types were observed in honey samples. 64% of the pollen types found in the samples were arboreal and 36% were herbaceous/shrubby. Analyzing the pollen spectrum of all samples, considering the nectar plants, it can be said that in this region, the honey of *A. mellifera* sp. is polyfloral and wild, and the species that most contributed to its formation were: *Alternanthera brasiliana* (L.) Kuntze (F. Amaranthaceae), *Mimosa arenosa* (Willd.) Poir. (F. Fabaceae), *Mimosa quadrivalvis* (L.) (F. Fabaceae), *Hyptis atrorubens* Poit (F. Lamiaceae), *Borreria alata* (Aubl.) DC. (F. Rubiaceae), *Borreria tenella* (Kunth) Cham. & Schltld. and *Borreria verticillata* (L.) G. Mey. It is recommended to preserve the native species found, important for the formation of honey.

### PP-327

#### Antioxidant capacity and potential therapeutic effect of monofloral and polyfloral honeys from the O'Higgins region of central Chile

Andrea Müller Sepúlveda<sup>1</sup>, Tamara Soto Araya<sup>2</sup>

<sup>1</sup>Instituto de Ciencias Agroalimentarias, Animales y Ambientales, Universidad de O'Higgins

<sup>2</sup>Escuela de Ciencias Agroalimentarias, Animales y Ambientales, Universidad de O'Higgins

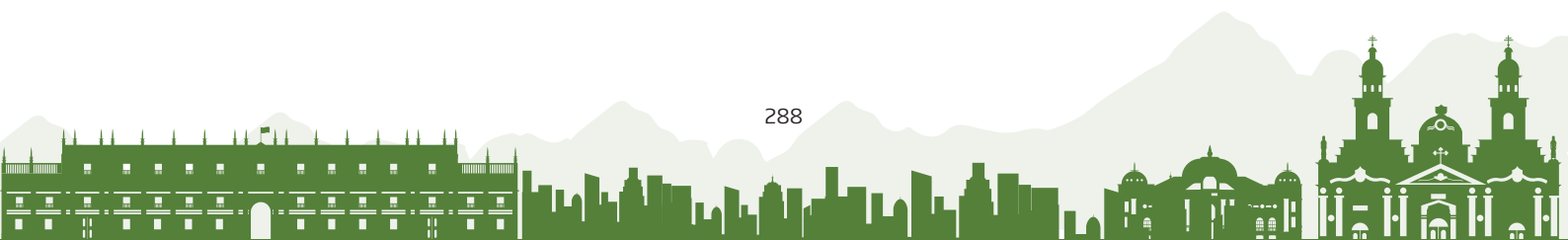
Honey has been used for medicinal purposes since ancient times due to the therapeutic properties conferred by the compounds present in it. These compounds include polyphenols, which vary according to their botanical and geographical origin and are related to honey's antioxidant capacity.

In order to determine the potential therapeutic effect through the antioxidant capacity, 41 samples of monofloral and polyfloral honeys from the O'Higgins region were collected. First, the concentration of total polyphenols was determined using the Folin-Ciocalteu colorimetric method. Subsequently, the antioxidant capacity was determined using the DPPH colorimetric assay (1,1-diphenyl-2-picrylhydrazyl radical assay). These results were compared and also related to their geographical location within the region.

No statistically significant differences were found in the samples of polyfloral honeys when compared to monofloral honeys ( $p < 0.05$ ). However, both analyses showed a higher concentration of total polyphenols and higher antioxidant capacity in the polyfloral honey samples compared to the monofloral honeys.

Correlating both analyses, our study showed a positive correlation between total polyphenol concentration and antioxidant capacity in honey samples from the O'Higgins region. Relating the results to geo-referencing, the commune in the O'Higgins region with the highest concentration of total polyphenols being statistically significant is Nancagua, while in the case of the comparison between provinces, there are no significant differences. The same occurs in the case of antioxidant capacity.

With these results, we can say that polyfloral honeys have a higher concentration of polyphenols and a higher antioxidant capacity, so they could have better medicinal properties. However, further studies are suggested to better understand this correlation, understanding that a larger number of honey samples and a greater homogeneity in the sampling in terms of the type of honey sample (monofloral versus polyfloral) are needed to obtain a greater representativeness in the study.



## Beekeeping Technology and Quality

### PP-328

#### Establishment of semi-automatic system for efficient royal jelly production

Hongmin Choi, Sangmi Han, Soonok Woo, Hyoyoung Kim, Sungkuk Kim, Seonmi Kim

Div. of Apiculture, Dept. Agricultural Biology, NAAS, RDA, Republic of Korea

This study was carried out to establish semi-automatic system for efficient royal jelly production. It is very important to establish a manufacturing process to improve the productivity of royal jelly prior to registration of health functional food ingredient. This system is a semi-automatic system using a machine and consists of 4 steps: making larvae plate, Transferring, larvae removal, and royal jelly collection. The conventional system showed an acceptance rate of 75%, and the semi-automatic system in this study showed an acceptance rate of 83.85%. Comparing the production of royal jelly, the production yields of royal jelly were  $4.17 \pm 1.2g$ ,  $47.1 \pm 2.7g$ , and  $39.4 \pm 1.4g$  in conventional system. In the semi-automatic production system,  $58.4 \pm 1.3g$ ,  $58.1 \pm 0.3g$ , and  $55.2 \pm 0.4g$  of royal jelly were produced. As a result of calculating the production efficiency for each system, the conventional method was 1.33g/min, and the semi-automatic system was 6.38g/min. The production efficiency using the semi-automatic system was 4.8 times higher than when using the conventional method. The content of 10-HDA in royal jelly produced using the two different systems, it was  $2.3 \pm 0.4\%$  for the conventional production system and  $2.2 \pm 0.3\%$  for the semi-automatic production system. No significant difference in content of 10-HDA was observed between two systems. Therefore, the semi-automatic system according to this study is expected to contribute to securing samples of royal jelly for registration of health functional food ingredient.

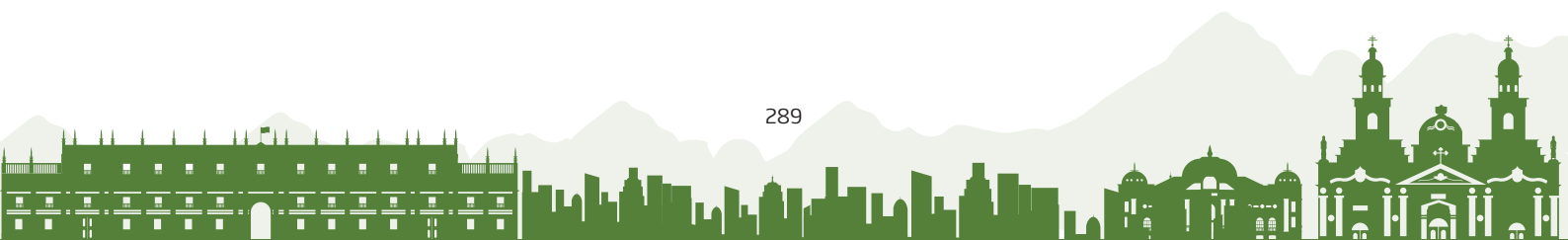
### PP-329

#### Phenolic Content In Monofloral Aroeira (*Astronium Urundeuva M. Allemão*) Honey From Caatinga Dry Forest, Piauí, Brazil

Juliana Nascimento Bendini, Márcia Maria Mendes Marques, Oliverton Escórcio De Oliveira, Ana Livia Sousa Campos

Federal University of Piauí, Picos, Brazil

Piauí State is the third largest honey producer in Brazil. Most of beekeepers are in the Caatinga Biome, also known as dry forest. The steppe savanna is the predominant vegetation whose adaptations to the semi-arid climate resulting in a rich biodiversity that is imprinted in the honey produced. *Astronium urundeuva M. Allemão* is a tree species that occurs in the Caatinga and that allows the production of monofloral honey, very appreciated as a medicine by the local people. This study aimed to quantify the phenolic compounds in monofloral honey from aroeira trees in a dry forest of Piauí State. Seven samples were collected from regional beekeepers. The botanical origin was attested using pollen analysis following the standard European methodology without application of acetolysis. Folin-Ciocalteu colorimetric method was used to determine the total phenolic content. It was observed that all samples showed significant values of total phenolic compounds, ranged from  $121.8 \text{ mg} \pm 5.09$  to  $181.8 \pm 2 \text{ mg}$  galic acid equivalentes/100g of honey sample. These results indicate that monofloral honey from the Piauí dry forest is rich in bioactive compounds, adding value to the product.





### PP-330 Monitoring the quality of Croatian honey

Nada Vahčić<sup>1</sup>, Saša Drakula<sup>1</sup>, Ksenija Marković<sup>1</sup>, Marina Krpan<sup>1</sup>, Sandra Pedisić<sup>2</sup>, Zoran Zorić<sup>2</sup>, Mirjana Hruškar<sup>1</sup>

<sup>1</sup>Department of Food Quality Control, Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

<sup>2</sup>Centre for Food Technology and Biotechnology, Faculty of Food Technology and Biotechnology, University of Zagreb, Zadar, Croatia

Three climate zones and the wealth of plant species in the Republic of Croatia enable the production of numerous types of honey. Therefore, the aim of this research was to analyze several physico-chemical quality indicators for 6 types of honey (acacia, chestnut, linden, flower, meadow and honeydew) over the past 10 years. Analyzed samples were included in the international honey quality competition in the Republic of Croatia "Zzzagimed" and refer to the seasons from 2013 to 2022.

Investigated physico-chemical parameters were: water content, electrical conductivity, free acidity, reducing sugars, sucrose and HMF content. Some of these parameters are the result of good production practices, while some are influenced by climatic factors and bee grazing. Results of the analysis show that values of mentioned parameters are mostly uniform, without major deviations. The water content ranged from 15.27% in honeydew to 18.23% in chestnut honey. The electrical conductivity is characteristic for the certain types of honey and averaged from 0.138 mScm<sup>-1</sup> for acacia to 1.328 mScm<sup>-1</sup> for honeydew honey. Free acidity reached the highest values in meadow and honeydew honey samples (27.15 and 27.62 mEqkg<sup>-1</sup>, respectively), while they were the lowest in acacia honey (average 11.86 mEqkg<sup>-1</sup>). The reducing sugars content ranged from 51.34% in honeydew to 70.57% in linden honey, while sucrose content was generally far below the maximum value allowed by national and/or international regulations for certain types of honey. The HMF content in all samples throughout the ten-year research period was minimal, up to 11.81 mgkg<sup>-1</sup> at most, which is a consequence of the fact that the honeys were sampled and analyzed within a period of up to 4 months after spinning.

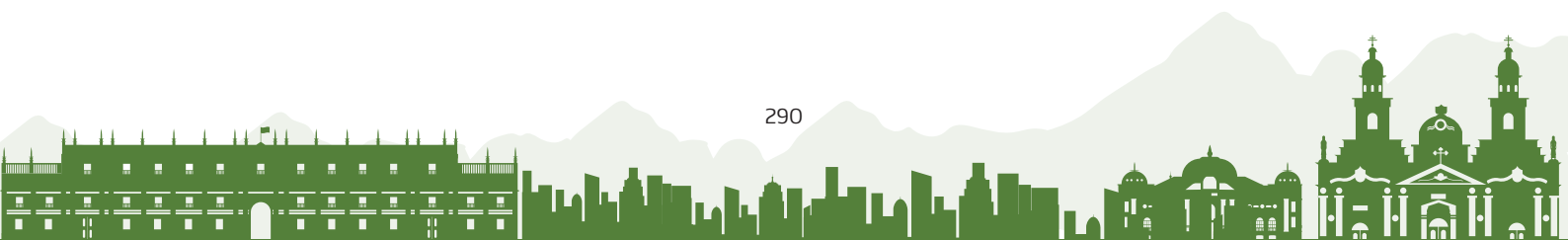
Analysis of variance showed that only sucrose and HMF content did not statistically significantly differ with respect to the type of honey, while only honey conductivity and acidity did not statistically significantly differ between seasons. PCA analysis showed that the botanical origin of honey cannot be reliably assessed based on the investigated parameters. Honey quality proved to be unquestionable throughout the entire ten-year research period.

### PP-331 Montpellier maple (*Acer monspesulanum* L.) honeydew honey: physicochemical properties, pollen spectrum and sensory profile

Dragan Bubalo, Lidija Svečnjak, Saša Prđun

Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, University of Zagreb Faculty of Agriculture, Zagreb, Croatia

The occurrence of significant amounts of honeydew on the Montpellier maple (*Acer monspessulanum* L.) in the sub-Mediterranean area of Croatia has been a frequent appearance since 2017. The aim of this study was to investigate the background mechanisms of the honeydew flow on Montpellier maple, and to characterize the corresponding honeydew honey by means of selected physicochemical analytical methods, as well as melissopalynological and sensory analysis. The study was carried out on 26 honeydew honey samples collected from Zabiokovlje area (Split-Dalmatia county). Prior to honeydew honey collection, the honeydew flow on Montpellier maple trees from the same area was monitored. Physicochemical analyses (determination of water content, electrical conductivity, and hydroxymethylfurfural - HMF), melissopalynological analysis and sensory analysis of collected honeydew honey samples were carried out in accordance with standardized methods. Initially, Montpellier maple honeydew was thought to be excreted, as is usual, by aphids, but the observations obtained in this study revealed that another insect species is responsible for the honeydew production. Namely, careful two-day monitoring of Montpellier maple trees during the honeydew flow revealed the presence of a leafhopper species determined as *Acericerus heydenii* Kirschbaum, 1868. The results of analysis of Montpellier maple honeydew honey revealed that an average water content was 14.5% (13.5 - 16.4%), electrical conductivity ranged from 1.50 to 2.01 mS/cm with an average of 1.77 mS/cm, while HMF content ranged from 0.0 to 5.8 mg/kg with an average of 2.5 mg/kg. Melissopalynological analysis showed that the ratio of honeydew elements and pollen of nectariferous plant species ranged from 1.17 to 7.55. The pollen grains of *Trifolium pratense*, *Cerinthe minor*, *Rhamnus* spp., *Melilotus* spp., *Allium* spp. and *Paliurus spina Christi* were determined in the pollen spectrum of analysed honey samples. Sensory analysis of Montpellier maple honeydew honey revealed a characteristic brown colour with a reddish tone, a medium strong odour and aroma of molasses, dried tomatoes and yeast extract, as well as light to medium sweetness with weak acidity. This preliminary study of Montpellier maple honeydew honey should be complemented with additional physicochemical parameters that will contribute to more detailed characterization of this honeydew honey type.





## Beekeeping Technology and Quality

### PP-332 Characterisation of monofloral bee pollen from Slovenia

**Aljaž Debelak**

Slovenian Beekeeper's Association, Lukovica, Slovenia

Within a three-year period, we gathered 60 samples of bee pollen from beekeepers all over Slovenia. In order to obtain monofloral samples we had to manually sort the pellets by the prevalent color within the polifloral samples gathered by the bees. We determined the botanical origin of the samples with the method of melissopalynology. In some cases, samples of similar botanical origin varied in color (pollen samples of *Acer* spp. were found in shades of green, brown, gray and yellow). All of the samples were then analyzed for the content of different elements, antioxidant and antimicrobial activities.

Pollen samples from *Hedera helix* exemplified the best antimicrobial activity against *S. aureus*. Some of the pollen samples from *Taraxacum* spp. exemplified good antimicrobial activity against *E. coli*. Pollen samples from *Brassica napus* exemplified good antimicrobial activity against *L. monocytogenes* and *C. jejuni*. Samples from *Castanea sativa* also showed good antimicrobial activity against *C. jejuni*.

Pollen samples from *Brassica napus* and *Castanea sativa* exemplified the best antioxidant activity measured by the DPPH method.

*Brassica napus* pollen contained the most calcium and sulfur. *Taraxacum* spp. pollen contained the most chlorine and bromine. *Castanea sativa* samples contained the most iron, manganese and zinc.

A sensory analysis of the samples was also performed by a four-member team experienced in food sensory analysis and sensory profiles for pollen samples of *Acer* spp., *Brassica napus* and *Taraxacum* spp. were formed.

The aim of this study is to form a database of the characteristics of pollen mentioned above and to compare Slovenian pollen with samples from studies from other parts of the world. With this knowledge consumers could implement monofloral pollen in their diets to help cater to specific needs.

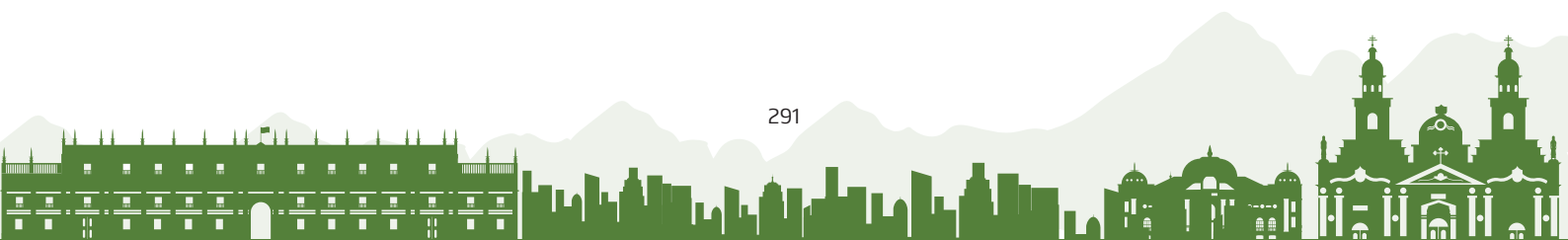
The results were produced within the framework of the National apiculture programme in the Republic of Slovenia in the years 2020-2022, which was financed from the state budget and the budget of the European Union.

### PP-333 Artificial intelligence for automated inspection of managed honeybee hives

**Joyce Almeida, Jorge Kavicki, Diego Moure-Oliveira, Carlos Pamplona Rehder, Guilherme Jorge Gomes Sousa, Andresa Berretta**

AgroBee, Ribeirão Preto, SP, Brazil

The automation of processes is a reality in several economic sectors, including agriculture. Technology has been changing the way of operating activities and all these advances will gradually make this sector more safe, efficient and sustainable. It is the period of the 4.0 agriculture. In beekeeping, 4.0 technology can be used to monitor hives, increase production, and ensure honey quality. An expressive bottleneck in the beekeeping activity is the revision of swarms after the nest division process. Newly divided swarms are weakened, and require specific handling aimed at rapid strengthening, observing sanity and posture pattern. The objective of this work was to develop an artificial intelligence (AI) to automate the inspection of managed honeybee (*Apis mellifera*) hives. The development of the digital tool occurred during 2020 and 2021, with a base data of 2,000 photos of brood frames, the tool was trained to identify closed brood cells, open brood cells and food cells, recognizing the posture and population pattern - strong or weak swarm. All the images used in the training were catalogued, and the result that the AI revealed was compared with the real evaluation. The DNN/CNN networks was used to the development of the AI. DNNs - Deep Neural Networks - are neural networks with multiple input and output layers and are able to find hidden trends in data, and CNNs are networks that use convolution layers, effective in tasks such as image detection and classification. Through an image analysis, the level 3 artificial intelligence recognizes and catalogs the health of the brood frames, with a sampling of 6 photos per beehive. At the end of the evaluation, the digital tool offers a note and recommendations according to the evaluation obtained - needs a queen change, needs protein food, needs energy food, etc. - offering more than an evaluation but a monitoring of that honeybee hive.



### PP-334 Analysis of Honey Bee Mating rate by Environmental factor and Phenology on Republic of Korea

**Bo-Sun Park**, Minwoong Son, Peter Njukang Akongte, Daegeun Oh, Dongwon Kim, Su Bae Kim, Su Jin Lee, Young Soo Choi

Apiculture Division, Department of Agricultural Biology, National Institute of Agricultural Sciences, Korea

The complicated aerial mating habit of honeybees has called for their breeding in isolated areas such as Islands to conserve and propagate genetic resources. We carried out breeding on Sik-do and Wangdeung-do Islands (Islands of the west coast) located in Korea and evaluated the rate of mating success within three years (From April 2020 to September 2022). Local factors did not influence mating rate, and the rate of mating success by season was highest in spring. The Sik-do Island is rich in food plants such as False Acacia (*Robinia pseudoacacia* L.) and Rapeseed (*Brassica napus* L.) while the Wangdeung-do Island is mainly dominated by Broad-leaf bamboo (*Sasa borealis*) (Hack, Makino and Shibata), which makes the vegetation monotonous compared to the Sik-do Island. However, the rate of mating success did not differ significantly in the two regions. It can be assumed that plant diversity has little or weak correlation with mating success rates. This study is expected to be used as basic data for breeding in Island regions through comparison of mating success rates at different climatic factors, land cover and flora.

### PP-335 Monitoring the residues of crop protection chemicals in bees' nests – case study from SE Poland

**Mikolaj Boranski<sup>1</sup>**, Aleksandra Splitt<sup>1</sup>, Jacek Jachula<sup>2</sup>

<sup>1</sup>Apiculture Division, The National Institute of Horticultural Research, Skierniewice, Poland

<sup>2</sup>Department of Botany, Mycology and Ecology, Institute of Biological Sciences, Maria Curie-Skłodowska University in Lublin, Poland

The study was conducted in 2022 in southeastern Poland in three apple orchards (*Malus domestica* Borkh). Two of them were managed conventionally (with standard chemical protection) and one was ecologically (without pesticides). In each of the three locations, colonies of honey bees (*Apis mellifera*), bumble bees (*Bombus terrestris*) and population of red mason bees (*Osmia bicornis*) were placed. We assayed five bee products for honey bees (uncapped nectar, honey, pollen, bee bread and wax), three for bumble bees (uncapped nectar, bee bread and wax) and one for red mason bees (larval provision). A total of 129 samples were tested for residues of plant protection products. Samples were taken at the end of blooming of apple orchards. We used a gas chromatograph (GC) and liquid chromatograph (LC) equipped with dual mass detectors (GC-MS/MS and LC-MS/MS) to detect residues of pesticides. Overall 50 residues of plant protection products or their metabolites were identified, among of them 11 substances which use is not permitted in the European Union. Of all the analyzed samples, only eight were found to contain no residues of pesticides. Most residues of plant protection products were detected in pollen from honey bee nests (in total 34 pesticides). The least contaminated product was uncapped nectar from bumble bee nests (6 residues of pesticides). In accordance with Regulation (EU) 396/2005 of the European Parliament and of the Council in 45% of the total number of samples maximum residue levels of pesticides (MRLs) were found to be exceeded. This study was possible through implementation of the research project DHR.bz.070.2.2022, task 6.5 entitled: "Monitoring the effects of plant protection on pollinating insects" financed by the Ministry of Agriculture and Rural Development, Poland.

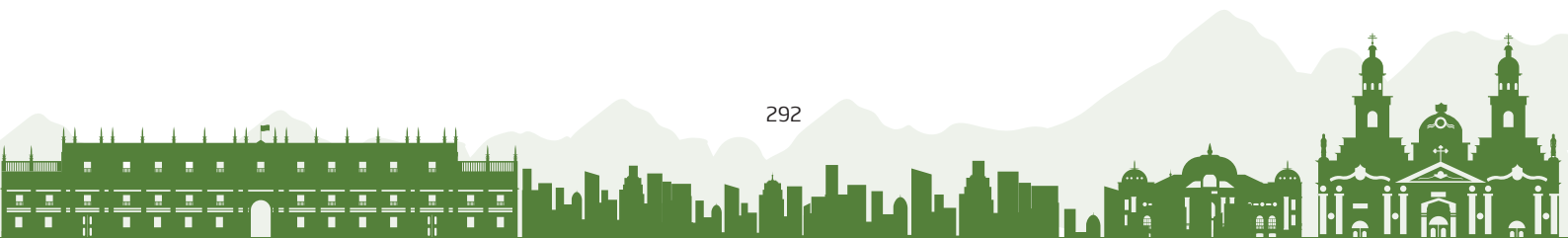
### PP-336 Physicochemical and microbiological characterization of honey from the south of the province of Santa Fe Argentina

**Milagros Rocío Lopez Hiriart<sup>1</sup>**, Leonel Matías Pérez Raymonda<sup>2</sup>

<sup>1</sup>Pérez Raymonda, Leonel, Sector Apicultura, Escuela Agrotécnica Libertador Gral. San Martín; Cátedra de Química Biológica, Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Casilda, Argentina

<sup>2</sup>López Hiriart, Milagros, Cátedra de Salud Pública, Facultad de Ciencias Veterinarias; Consejo de Investigaciones de la UNR, Universidad Nacional de Rosario, Casilda, Argentina

The production of honey in Argentina conforms a traced productive process, whose main raw material is exported. Occupying one of the main producing and exporting countries of honey in bulk worldwide. The province of Santa Fe, is among the main beekeeping jurisdictions in the country, both in number of producers, hives and production. The objective of this work was to characterize, through physicochemical and microbiological analysis, honey obtained in a community honey extraction room located in the south of the province of Santa Fe. 65 honey samples from 7 departments in the southern province were analyzed. which were processed in a municipally-managed community extraction room, during the years 2021 and 2022. The physicochemical analyzes were carried out according to the Guide for the Characterization of Argentine Honeys. For microbiological analysis Fungi and yeasts were analyzed according to the APHA Standards, coliforms according to ICMSF Standards and *Salmonella* sp., using CHROMagar TM. The results of the physicochemical parameters obtained showed that the free acidity of some samples is above the maximum value allowed in the Argentine Food Code (CAA) (40 meq /Kg). Regarding the pH, all the samples were within the expected values (3.2 and 4.5). Regarding the % moisture and % total solids, some samples are above the maximum allowed. In relation to hydroxymethylfurfural (HMF), all the values were within the current regulations, which place 40mg/Kg of HMF as the maximum value. In the color parameter they covered a spectrum of shades, ranging from white (17/34 mm Pfund) to dark amber (114/140 mm Pfund), which demonstrated the diversity of the bee flora in the apiaries of origin. Regarding the analysis of glyphosate and AMPA, 24 samples were selected according to the location of the apiaries, finding only four samples outside the maximum parameter (50 ug / kg), established by the European Union. In conclusion, the physicochemical and microbiological characterization of honey from the Santa Fe region is important not only for food safety but also for international marketing of the product.



### PP-337

#### Quality of royal jelly produced by Africanized *Apis mellifera* in southern Brazil

Alessandra Benites<sup>1</sup>, Douglas Galhardo<sup>1</sup>, Cibele Regina Schneider<sup>1</sup>, Jessica Carolina Camargo Lopez<sup>1</sup>, Wendy Guadalin De Oliveira<sup>2</sup>, Daiani Rodrigues Moreira<sup>3</sup>, Vagner deAlencar Arnaut de Toledo<sup>4</sup>

<sup>1</sup>Postgraduate Program in Animal Science, State University of Maringa, Maringa, Brazil

<sup>2</sup>Postgraduate Program in Animal Science, Federal Technological University of Parana, Dois Vizinhos, Brazil

<sup>3</sup>Department of Biotechnology, Genetics and Cell Biology, State University of Maringa, Maringa, Brazil

<sup>4</sup>Professor at the State University of Maringa, Animal Science Department and Postgraduate Program in Animal Science

Royal jelly demands a higher technical level from the beekeeper for its production, as well as greater investments in management, feeding the colonies and the structure of laboratories for production and conservation. The objective of this research was to evaluate the quality of royal jelly production in southern Brazil. The research was carried out at the Laboratory of Beekeeping and Meliponiculture, belonging to the State University of Maringa. The royal jelly production period was carried out between June 2019 and May 2020, totaling twelve months of collections. The colonies were managed and standardized, obtaining colonies in the nest and on the nest. Royal jelly production was produced by supplementing only with 600 mL of 50% sugar syrup. After collecting the royal jelly, the samples were stored in a freezer at  $-18\text{ }^{\circ}\text{C}$ . Royal jelly physicochemical measurements were performed such as moisture (%), ash (%), acidity (mg NaOH/g), protein (%), lipids (%). The data were evaluated by grouping the samples in the four seasons of the year, winter, spring, summer and autumn. The average moisture content was  $68.21\pm 2.1\%$ , however, when comparing the samples grouped by the seasons of the year, they showed differentiation ( $p < 0.05$ ), in which the winter samples had a higher content in relation to the samples of spring and summer. Ash content averaged 1.37%, with no difference ( $p > 0.05$ ) between samples. Royal jelly acidity averaged 49.52 (mg NaOH/g), differing only between summer and winter. The average protein value was 14.08%, with differentiation ( $p < 0.05$ ) between the samples from the four seasons. The average lipid content (4.59%), being within the limits observed by the regulations, however, there was no difference ( $p > 0.05$ ) between the seasons and the months of collection. It is concluded that the observed contents of the samples do not differ between them, all values correspond to the established by national and international regulations on the quality of royal jelly and can be used for future studies on the seasonality in beekeeping production.

### PP-338

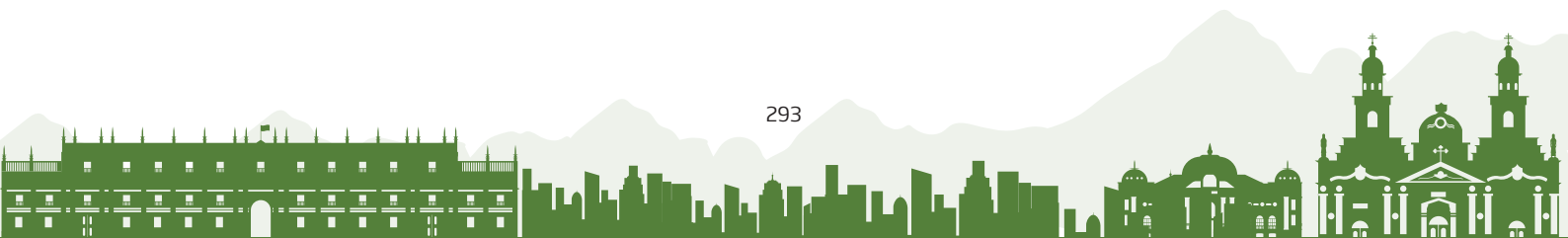
#### Honeybee waterer - Low-cost technology that improves the productive efficiency of the colonies

Tânia Patrícia Schafaschek<sup>1</sup>, André Sezerino<sup>2</sup>

<sup>1</sup>Agricultural Research and Rural Extension Company of Santa Catarina, Videira Experimental Station, Videira-SC, Brazil

<sup>2</sup>Agricultural Research and Rural Extension Company of Santa Catarina, Caçador Experimental Station, Caçador-SC, Brazil

Water is used by bees in their metabolism and other functions in the colony. In the absence of water sources, it is essential to provide a water station. In this work we present the making of a waterer and the preliminary results of the evaluation of the visitation for the collection of water. The waterer is made up of a high density polyethylene and high molecular weight (HDPE) drum with a capacity to store 50 liters of water with a screw cap and a 100 mm PVC tube with a cut at the top. A 25 mm threadable and weldable adapter is used at the bottom of the lid of the drum, attaching, in sequence, a 25 mm PVC pipe with an approximate measure of 40 cm. At the other end of the 25 mm PVC pipe, a 25 mm weldable/threadable  $90^{\circ}$  elbow is attached, a 20 mm reducer and a 20 mm flexible coupling, which is connected to another adapter with a threadable flange, fixed to the 100 mm PVC pipe. In this tube, a cut is made in the upper part and the ends are sealed with CAPS PVC 100 mm. At the end where there is a connection with the pipe, coming from the water tank, an empty space is left and a  $\frac{1}{2}$  float tap is installed. The inside of the tube is then filled with washed sand. We evaluated water stations which were installed in an apiary and in an orchard cultivated under anti-hail screens. The number of bees arriving at the waterer per minute and their visitation time were evaluated. The bees constantly visited the waterer in both environments, including in climatic conditions considered unsuitable for flying and foraging. The installation of the water station in the orchards can be an option to improve the permanence of the bees under the screen and preventing them from going out in search of water. The availability of easily accessible waterer for the bees in the apiary can provide significant gains for the colony, thereby ensuring energy savings in collection and increasing the longevity of the bees.



### PP-339

#### Identification of the Botanical and Geographical Origin, Physicochemical Parameters and Organoleptic Characteristics in Monofloral and Bifloral Samples of Examined Chilean Honey

Patricio Vásquez Quitral<sup>1</sup>, Andrés Vargas Fernández<sup>2</sup>, Nadia Ríos Miranda<sup>3</sup>, Eduardo Castañeda Zúñiga<sup>4</sup>, Pablo Ulloa Fuentes<sup>5</sup>, Héctor Carrasco Altamirano<sup>6</sup>, Andrés Olea Carrasco<sup>6</sup>

<sup>1</sup>Escuela de Medicina Veterinaria y Agronomía, Universidad de Las Américas, sede Providencia, Santiago, Chile.

<sup>2</sup>Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile, Avda. Santa Rosa 11315, La Pintana, 8820808 Santiago, Chile.

<sup>3</sup>Departamento de Producción y Sanidad Vegetal, Universidad Austral De Chile, Valdivia, Chile.

<sup>4</sup>Instituto Profesional DUOC-UC, sede Puente Alto, Santiago, Chile.

<sup>5</sup>Laboratorio De Postcosecha, Instituto Nacional de Investigaciones Agropecuarias (INIA) La Platina, La Pintana, Santiago, Chile.

<sup>6</sup>Instituto De Ciencias Químicas Aplicadas, Facultad De Ingeniería, Universidad Autónoma De Chile, San Miguel, Santiago, Chile.

To contribute to the Chilean honey's characterization, 30 samples were analyzed by using comprehensive physicochemical, pollen and sensorial analyses. For that, local beekeepers donated honey samples from different geographical locations: Cajón del Maipo, Casablanca, Choshuenco, Collipulli, Curanilahue, Curicó, El Buchén, Frutillar, Llifén, Mallarauco, Parral, Pichidegua, Pirque, Puerto Varas, among others. Physicochemical parameters required for honey authentication such as: color (28–71° Pfund scale), concentration (42,20–43,25 °Bé), density (1,41–1,43 Kg/L), electrical conductivity (0.25–1.47 mS/cm), humidity (14.4–16.9%), pH (3.8–4.7), and soluble sugars (79,50–81,50 °Brix) were determined by conventional techniques in all samples. In addition, in the monofloral and bifloral honey samples, the diastase activity (5,7–33,2 °G), the hydroxymethylfurfural content (0,11–2.56 mg/Kg) and the sensorial properties were determined after that the palynological analysis were performed. Bifloral, monofloral and multifloral Chilean honey samples analyzed showed physicochemical properties in normal ranges for natural honey, according to the national and international food regulation; which confirmed their authenticity and high quality. Also, this work describes for first time the preliminary sensorial properties of 15 monofloral and bifloral Chilean honeys samples.

### PP-340

#### Phenolics from Chilean bee bread exhibit antioxidant and antibacterial properties

Paula Nunez-Pizarro<sup>1</sup>, Bairon Jorquera<sup>2</sup>, Adriano Costa De Camargo<sup>3</sup>, Gloria Montenegro<sup>4</sup>, Raquel Bridi<sup>2</sup>

<sup>1</sup>Doctorado en Ciencias de la Agricultura, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>2</sup>Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

<sup>3</sup>Nutrition and Food Technology Institute, University of Chile, Santiago, Chile

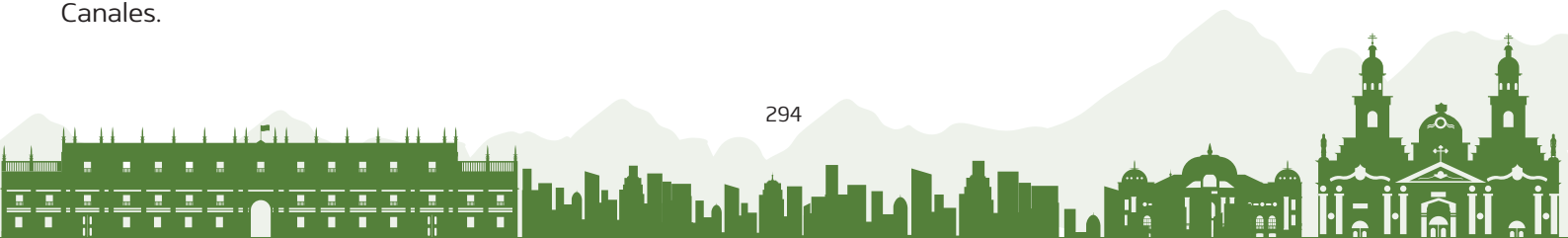
<sup>4</sup>Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile

Bee bread (BB) is a natural product from the beehive obtained from the fermentation of bee pollen combined with flower nectar and bee saliva. The chemical analysis of bee bread revealed that it is typically made up of water, proteins, free amino acids, carbohydrates, fatty acids, and other bioactive molecules; however, the composition varies from region to region depending on the melliferous plants present there as well as the local climate and seasonal variations. Research groups have outlined several potential bioactive roles for honeybee pollen, including antioxidant, antitumoral, hepatoprotective, and antimicrobial activities. The potential application of beebread is related to its nutritional and functional components, including phenolic compounds.

To fill an existing literature gap, the palynological analysis, phenolic composition, antioxidant activity, and antibacterial properties of a Chilean bee bread are reported for the first time.

The tested material exhibited high levels of phenolic compounds by Folin-Ciocalteu's method (1340 ± 186 mg GAE/100 g BB) and showed antioxidant capacity using ferric-reducing antioxidant potential (FRAP) (51 ± 2 µmol Trolox equivalent/g BB) and Oxygen Radical Absorbance Capacity (ORAC-FL) (643 ± 64 µmol Trolox equivalent/g BB). Phenolic acids and flavonoids were identified by UPLC-MS/MS. Furthermore, ferulic acid, quercetin, apigenin, rutin and kaempferol were the major phenolic compounds present in the methanolic extract. The extract showed mild activity against *Staphylococcus aureus*, but it was particularly effective in inhibiting *Streptococcus pyogenes* giving a minimum inhibitory concentration (MIC) value of 0.976 mg/mL. This study demonstrates the bioactive potential of Chilean bee bread and supports the idea that this bee product is a promising source of antioxidants and antimicrobial compounds.

Acknowledgments: The authors gratefully acknowledge support from the associated beekeeper, Raúl Antonio Rojas Canales.





### PP-341

### Honeybee pollen from southern Chile: phenolic profile, antioxidant capacity, and bioaccessibility

Paula Nuñez Pizarro<sup>1</sup>, Arturo Larena<sup>2</sup>, Gabriel Núñez<sup>3</sup>, Gloria Montenegro<sup>3</sup>, Raquel Bridi<sup>4</sup>

<sup>1</sup>Doctorado en Ciencias de la Agricultura, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>2</sup>Departamento de Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>3</sup>Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>4</sup>Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

Honeybee pollen (HBP) chemical composition is highly variable according to the floral and geographical origin of the pollen grains. The beneficial effects and functional properties of HBP are well-known and have been mainly attributed to their high content of antioxidant polyphenols. In this work, twelve HBPs samples from the southern region of Chile (X Región de Los Lagos) were characterized for the first time according to their botanical origin, phenolic composition, and antioxidant activity. The *in vitro* gastrointestinal digestion assay was done to simulate the human upper digestive tract. Selected honeybee pollen extracts (HBPEs) were assessed as bioaccessible fractions during an *in vitro* gastrointestinal digestion. Contents of phenolic compounds, antioxidant capacity, and recovery index of quercetin, myricetin, and cinnamic acid were monitored in different steps of gastrointestinal digestion.

The introduced species *Brassica rapa* L. (Brassicaceae), *Lotus pedunculatus* Cav. (Fabaceae), and *Ulex europaeus* L. (Fabaceae) predominated in all the HBPs analyzed, while the native species *Buddleja globosa* Hope (Scrophulariaceae), *Luma apiculata* (DC.) Burret (Myrtaceae), *Embothrium coccineum* J.R. Forst. & G. Forst. (Proteaceae) and *Eucryphia cordifolia* Cav. (Cunoniaceae) appeared less frequently. The results showed significant phenolic content, antioxidant capacity, and reducing power. Cinnamic acid, myricetin, and quercetin had high concentrations in all samples. All samples showed antibacterial activity against *Streptococcus pyogenes*. The content of polyphenols and antioxidant capacity in HBPEs achieved full bioaccessibility at the end of the intestinal digestion step. However, results obtained using HPLC-DAD demonstrated relatively low values of bioaccessible quercetin and cinnamic acid after the digestion process. In contrast, myricetin showed high bioaccessibility in the intestinal digestion steps.

In conclusion, HBPs from the X Region de Los Lagos have been found to exhibit antioxidant and antimicrobial activities and should be further considered a promising candidate for classification as functional foods.

### PP-342

### Palynological analysis, chemical characterization and antibacterial activity of sixteen bee pollen from different regions of Chile

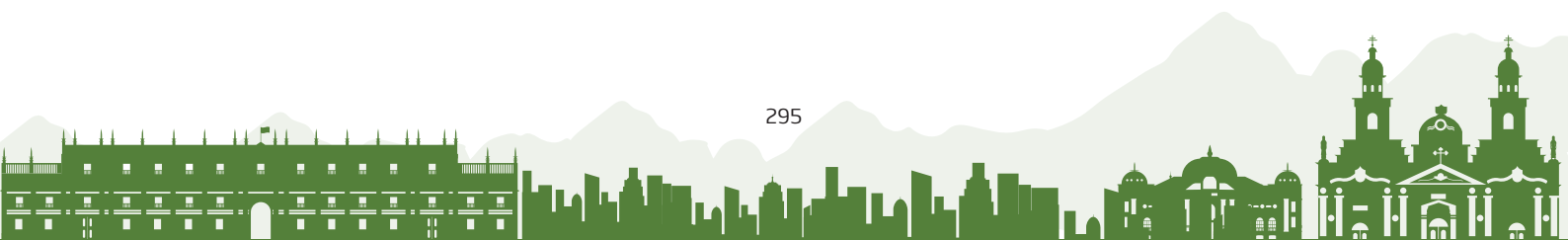
Enrique Alonso Rojas<sup>1</sup>, Juan González<sup>1</sup>, Valentina González<sup>1</sup>, Karen Yáñez<sup>1</sup>, Daniel Ramírez<sup>2</sup>, Beatriz Camara<sup>1</sup>, Jean Pierre Froncoise<sup>3</sup>

<sup>1</sup>Centro de Biotecnología Dr. Daniel Alcalay Lowitt, Universidad Técnica Federico Santa María, Av. España 1680, Valparaíso, Chile.

<sup>2</sup>Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Av. España 1680, Valparaíso, Chile.

<sup>3</sup>Laboratorio de Geografía Ambiental y Palinología, Universidad de Playa Ancha, Leopoldo Carvallo 270, Playa Ancha, Valparaíso, Chile.

Corbicular pollen is a mixture of collected pollen and salivary secretions that the bee kneads into small balls and transports on the third pair of hind legs to the hive, where it is collected by beekeepers with pollen traps before the bees enter through the entrance. It is well known that bee pollen has a high nutritional value, since it contains proteins, carbohydrates, essential amino acids, vitamins and minerals, nutrients used not only by the hive, but also by man, who increasingly consumes this product as a food supplement, which is also a potent antioxidant, whose high content of polyphenols and flavonoids, gives it antibacterial and antifungal properties. This study presents the palynological analysis to determine the botanical origin of sixteen samples of fresh frozen pollen applying the Chilean Norm (NCh 3255, 2011). The antibacterial activity of pollen extracts was studied by agar diffusion assay method using Gram-positive strains *Listeria monocytogenes* (07PF0776). In total, 9 of the 16 extracts evaluated showed activity. Of this set, the pollen extract from Villarica, Araucanía region (M15), showed the highest activity against *L. monocytogenes* ( $7.5 \pm 2.2$  mm), evidencing the great bioactive potential of these bee pollen samples against human pathogenic bacteria. Additionally, some physical and chemical properties (total phenols, pH value and humidity) were determined to differentiate and valorize the fresh pollens from different geographical areas of Chile.





### PP-343

#### Local precipitation changes and migratory beekeeping from Central Chile forced by aridity increase are the main concerns of beekeepers from northern Patagonia

Valentina Lepe<sup>1</sup>, Duncan Christie<sup>1</sup>, Carla Marchant<sup>2</sup>, Robin Browaldh<sup>1</sup>, Ariel Muñoz<sup>3</sup>, Martina Gajardo<sup>3</sup>, Karen Yañez<sup>4</sup>

<sup>1</sup>Instituto de Conservación Biodiversidad y Territorio, Universidad Austral de Chile, Valdivia, Chile

<sup>2</sup>Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia, Chile

<sup>3</sup>Instituto de Geografía, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

<sup>4</sup>Centro de Biotecnología, Universidad Técnica Federico Santa María, Valparaíso, Chile

The severity of the current Megadrought affecting Mediterranean Central Chile uninterruptedly since 2010 and to a lesser extent the neighboring rainy Patagonia, has had widespread impacts on beekeeping through direct effects with a severe restriction in the availability of flowers and nectar especially in Central Chile, and indirect effects on neighboring regions derived from the migratory beekeeping to Patagonia. Several studies worldwide have shown that migratory beekeeping can have varied effects on the health and genetic diversity of honeybees and wild bees, but little is known about this phenomenon forced by climate change. In this paper we show the results for Chilean Patagonia (>39°S) of the first national diagnosis about the impacts of climate change on beekeeping activity. Surveys were carried out to more than XXX beekeepers belonging to the regions of Los Ríos, Los Lagos and Aysén, which present a mostly Temperate climate; The perceptions of beekeepers about the effects of climate change on their activity and the adaptation practices they have carried out were evaluated. Our results indicate that the regional precipitation decline and the massive migratory beekeeping from central Chile to rainy Patagonia practiced as a adaptation measure to face the aridity increase Megadrought, emerges as the main regional concerns of beekeepers from Chilean Patagonia regarding climate change threats in south-central Chile.

The beekeepers of Patagonia indicate the need for the state to regulate migratory beekeeping to avoid possible negative effects on their honey production in addition to local drought, due to socio-environmental conflicts arising from increased floral competition, exceedance the carrying capacity of local ecosystems, and transmission of diseases. On the other hand, land use changes, the degradation of native forests, and rural-urban migration would have relevant effects on the continuity of beekeeping at local family farming. The perceptions of the beekeepers reinforce the need to advance with the development of socio-environmental research on beekeeping and associated socio-ecosystems in Patagonia. The development of a timely regulation of migratory beekeeping emerges a as a basis for the continuity of rural activity threatened by the future regional increase in aridity changes.

### PP-344

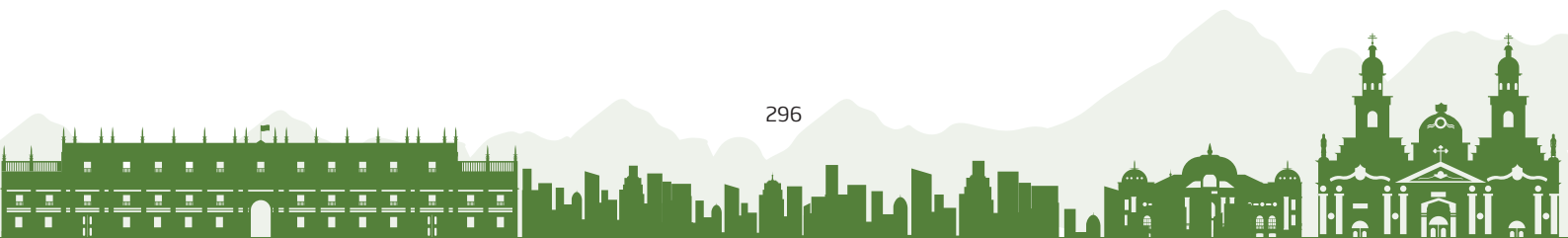
#### Beekeeping Architecture: An essential cornerstone for IoT in Beekeeping

Fulvio Lizano Madriz<sup>1</sup>, Alberto Sáenz Coto<sup>2</sup>

<sup>1</sup>Escuela de Informática, Universidad Nacional, Heredia Costa Rica

<sup>2</sup>Pollen Keepers, San José, Costa Rica

Manny of modern hive architecture elements are based on Lorenzo Lancstroth and François Huber proposals to implement the "bee space" and to facilitate beekeeping management and production. However, this design does not contemplate the use of information technology (IT) elements present today in many agro-industrial development contexts. These IT elements are part of what is known as the Internet of Things (IoT). IoT is defined as the set of scenarios where the connectivity of communication networks and computational capacity are extended to objects, sensors and everyday items that are not normally considered computers. IoT allows to these devices to exchange and consume data with minimal human intervention. The introduction of IoT elements in the modern hive inevitably goes through an architectural redesign. By using IoT, these devices can exchange and consume data with minimal human intervention. The introduction of IoT elements in the modern hive inevitably goes through an architectural redesign. Beekeeping Architecture (AA) is a development and research emergent field to incorporate IoT elements within the hive in the most efficient and effective way possible. In addition, AA is conceived as a research process to allow having data that makes it possible to identify potential problems as well as eventual causes of deterioration of the bee colony. AA allows to give voice to the materials, structures, and the colony. Several studies in the literature address the inclusion of IoT in the hive. However, these studies do not include information related to AA. In this presentation we show the progress of ongoing AA projects in aspects such as the development of an automatic trap for pollen collection and birth control, as well as the introduction of IoT elements for the recording and subsequent analysis of the internal sounds of Beehive.



### PP-345

#### Rapid determination of adulterant sugars from Raman spectroscopy

Felipe Leyton Soto<sup>1</sup>, Gloria Montenegro<sup>2</sup>, Ady Giordano<sup>1</sup>, Mauricio Isaacs<sup>1</sup>

<sup>1</sup>Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>2</sup>Facultad de Agronomía e Ingeniería Forestal Pontificia Universidad Católica de Chile

Honey is a natural product generated by bees that is recognized throughout the world for its high nutritional value and the multiple beneficial effects it provides. However, the honey is commonly adulterated by cheaper, commercially available sugar syrups. Thus, the search for fast, simple, and cheap analytical methodologies for determining adulteration in food matrices is essential such as Raman spectroscopy. In this work, the potential of Raman spectroscopy to determine the typical spectra of honey and its authenticity was evaluated, coupled with chemometric algorithms, PCA and SIMCA, and PLS modelling. Different honey samples from Chile were adulterated by solutions of glucose, fructose, sucrose, and commercial syrup (10–90%). In unadulterated honey, characteristic signs of vibrations associated with carbohydrates were observed in the footprint area between 200–600  $\text{cm}^{-1}$ ; we can find deformations related to groups of saccharides C-C-C, C-C-O, C-C, and C-O. On the other hand, the results show that the combination of spectroscopic information with qualitative chemometric algorithms (PCA and SIMCA) allowed classifying or discriminating of the nature of the honey samples studied. Based on its authenticity, the classification errors ranged between 3.3 and 10.8% for each group of samples. The data of quantitative analysis indicate that PLS models developed in this work, can be used efficiently for adulteration detect, where prediction errors between 3.12 and 6.34% were obtained for the syrups under study. Therefore, Raman spectroscopy surges as a fast alternative to classify and quantify the adulterant content in honey samples at the screening level since it provides a unique vibrational fingerprint to identify a molecule.

### PP-346

#### Evaluation of physical-chemical properties of propolis from the northwest of the Dominican Republic

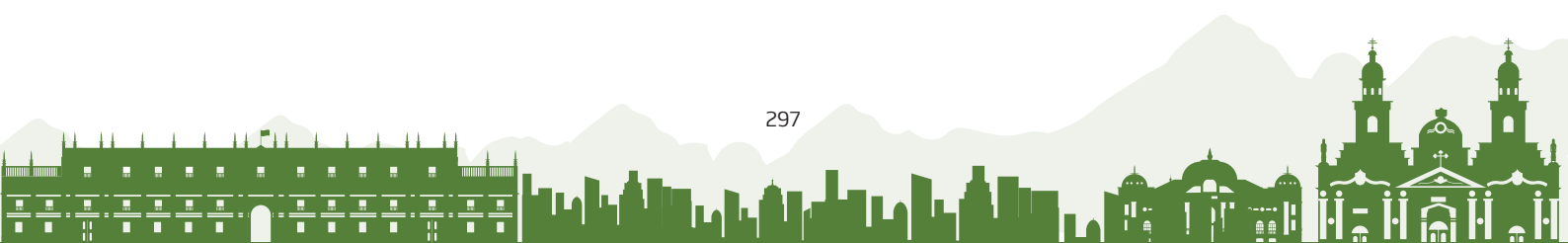
Ashley Guzmán<sup>1</sup>, Yaset Rodríguez Rodríguez<sup>1</sup>, Angel Camejo<sup>2</sup>, Sesar Rodríguez<sup>3</sup>

<sup>1</sup>Instituto Tecnológico de Santo Domingo, Dominican Republic

<sup>2</sup>LabStore, Santo Domingo, Dominican Republic

<sup>3</sup>Consortio Ambiental Dominicano, Santo Domingo, Dominican Republic

This research evaluated the physical-chemical properties of propolis of Dominican origin collected in three apiaries in the northwest of the country, as part of an initiative to provide information on their properties, their chemical composition and for their classification according to their ecosystem. originally. For this, the following physicochemical parameters were evaluated: humidity, ash content, waxes, resins, mechanical impurities, oxidation time, total content of phenols and flavonoids. The representative samples of dry forest, humid forest with grasses and mangroves presented humidity values of  $1.33\% \pm 0.07$ ,  $1.53\% \pm 0.06$  and  $0.70\% \pm 0.70$ , respectively, ashes of 3.37%, 1.08% and 2.06%, waxes of 36.74%  $\pm$  oxidation time of  $12.5s \pm 0.7$ ,  $44.5s \pm 0.7$  and  $17s \pm 1.4$ . These values correspond to the environment of the sampling area and the conditions of their collection. In the superficial analysis of the ashes through scanning electron microscopy no heavy metals were found. Hydroalcoholic and soft extracts of propolis were elaborated to replicate the products available in the market. The biological properties attributed to propolis depend on the content of phenols and flavonoids in them. They presented values of phenolic compounds and flavonoids within the parameter of the IRAM standard. When these contents were analyzed in the samples, we obtained favorable results that could enhance this product for its use at an industrial level.



### PP-347

#### Prebiotic potential of by-product after supercritical fluid extraction from bee pollen from high mountains of the Colombian Andes

Consuelo Díaz-Moreno<sup>1</sup>, Camila Bernal-Castro<sup>2</sup>

<sup>1</sup>Instituto de Ciencia y Tecnología de Alimentos (ICTA), Universidad Nacional de Colombia, Bogotá, Colombia

<sup>2</sup>Facultad de Ciencias, Doctorado en Biotecnología. Universidad Nacional de Colombia, Bogotá, Colombia

Bee pollen from *Apis mellifera* is a natural food with high nutritional value and biological activity, collected in the Colombian Eastern Cordillera (~2000–3000 m above sea level), with functional characteristics and originating from sustainable production. The study included 3 regions of different botanical and geographical origins from Guatavita, Mosquera, and Usme. Bee pollen samples were subjected to supercritical fluid extraction, obtaining an extract rich in carotenoids and solid residue source of protein, fiber, carbohydrates, micronutrients, and antioxidants. It is considered a suitable substrate for the growth of probiotic microorganisms. The solid residue was used to evaluate the in vitro prebiotic capacity through the ability to promote the growth of a mixed culture VEGE 092 from the Danisco® HOWARU® commercial line and axenic culture of *Pediococcus pentosaceus*, a strain recently isolated from a sample of bee bread to assess its growth. As control carbohydrates, analytical grade lactose, and Orafiti® P95 were used as commercial prebiotics with scientific evidence. The prebiotic index was determined through plate counts in MRS agar (Oxoid®) at 0 and 24 hours of incubation under microaerophilic conditions at 37°C. In addition, the prebiotic activity was evaluated by co-cultivation with a strain pathogenic *Escherichia coli* ATCC 25922, through the viability of pathogenic strain and probiotic strains independently by plate count on MRS agar (Oxoid®) for lactic acid bacteria (LAB) and on MacConkey agar (Oxoid®) for enterobacteria 0 and 24 hours at 37°C incubation. The results showed in vitro prebiotic index, indicating that the solid residues of pollen evaluated could be considered a carbon source for LAB, because it had a value equal to and higher than that of the control carbohydrate (lactose). In the prebiotic activity, a decrease of more than two logarithmic units was observed after 24 h of fermentation in the co-culture of *P. pentosaceus* and *E. coli* in the presence of the sample of the solid residue of pollen from the Mosquera region. This sample demonstrated the ability to promote the selective growth of the two probiotic strains evaluated in this study, in addition to inhibiting the growth of the pathogenic strain.

### PP-348

#### Granulated honey: From undesired product to winner of WBA Worlds Best Honey 2017

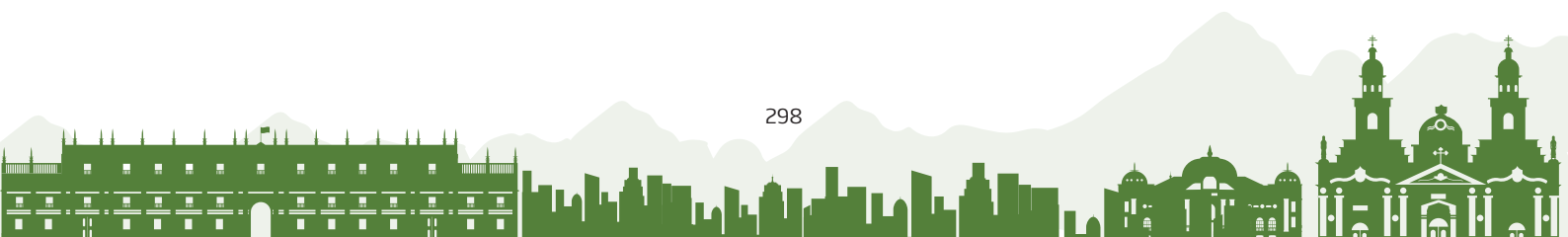
Lars Kromann Fischer

Beekeeper in Denmark

In 2017, at the Apimondia Congress in Istanbul, we won the WBA honey contests and received the Carl and Virginia Webb Silver Bowl for the World's Best Honey. The trophy winning honey was a creamed Danish spring honey predominantly based on nectar from rapeseed, hawthorn and stone fruits. It is the first time a granulated honey won this prestigious title. We will use our spring honey as case study for this talk.

In this talk we will give an overview of what happens when a honey crystalizes and which tools we have at hand to influence and control this process. More specifically, we will talk as seeding as a tool to control crystallization. We take a quick look at which factors induce and enhance crystallization and which factors inhibit and delay crystallization. Water content, temperature and the amount of crystal seeds in the honey are important parameters for the dynamics of crystal growth and thereby also for the quality of the final product.

Granulated honey is an exciting niche product for beekeepers in many parts of the world. It is a delicious food source which often stands out from the other types of honey at the shelf or at the market.



### PP-349

#### Exploring the Physico-Chemical, Functional, Sugar and Amino acid Profile of Indian Thyme Honey

Vikas Nanda, Dilpreet Singh Brar

Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal, Punjab, India

Indian Thyme honey samples (n=100) were collected from four different locations, namely Chittorgarh (D1), Jaipur (D2), and Partapgarh (D3) of the state Rajasthan and Neemuch (D4) district of Madhya Pradesh. The results of melissopalynology depict the pollen count was more than 80% which authenticates the botanical origin of the honey variety. The results of physicochemical analyses highlight the uniqueness of Indian thyme honey compared to thyme honey from other countries. The colour value of Indian thyme honey ranged between 95 to 105 Pfund, which was quite higher than the honey produced in other nations. The values of other parameters like pH ranged from 3.87 to 4.01; moreover, HMF content, DN (diastase number), moisture and ash lay between 8.62 to 15.23 mg/kg, 22.90 to 25.80 DN, 18.71 to 19.59%, 0.18 to 0.20% respectively. Sugars analysed were glucose, fructose, sucrose, turanose, maltose, gentiobiose and raffinose, from which fructose was the primary sugar which ranged between 35.64 to 40.91 g/100g and raffinose (0.11-0.32 g/100 g) was minor sugar compared to other sugars. The glucose-to-fructose ratio was 0.99 to 1.10. Besides, the sucrose content in the honey from all geographical locations was documented from 0.64 to 1.54 g/100 g. Additionally, proline, Phenylalanine, Tyrosine, Valine, Leucine, Glutamine, and aspartic acid amino acids were analysed in all samples, from which proline was the major amino acid reported with a range of 635 to 846 mg/100 g while alanine (26-47 mg/100 g) was present in quite a small amount. At the same time, aspartic acid (109-127 mg/100 g) and glutamine (117-168 mg/100 g) were other highly occurring amino acids after proline in Indian thyme honey. Besides these parameters, the antioxidant activity of the honey from all four geographical locations was quite impressive. The % antioxidant activity by DPPH assay ranged from 69.97 to 75.27 %. In addition, the total phenolic content (TPC) ranged between 58.22 – 73.82 mg GEA/100 g, so, these values conclude the high functional value of the Indian thyme honey.

### PP-350

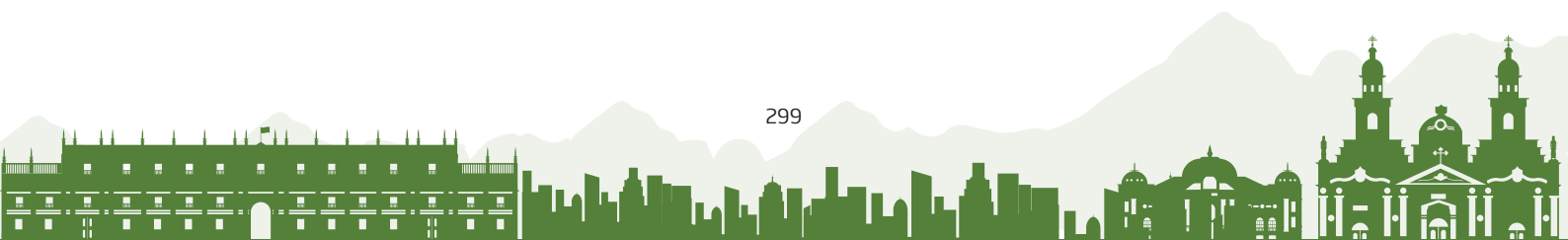
#### Study of the applicability of propolis extracts in the biogenic synthesis of magnetic nanoparticles

Carmen Ofelia Galvis, Christian Fabian Varela, Carlos Arturo Parra

Universidad Pedagógica y Tecnológica de Colombia

In the present work, the physicochemical characterization of samples of propolis from the municipalities of San Mateo, Pesca and Rondón in the department of Boyacá was carried out, to evaluate their quality based on the Cuban branch standard 932-88, this quality is determined in percentages of each of the samples, the regulations establish an acceptance or rejection if its value is within the range. In this regard, the samples satisfactorily complied with the established parameters, except for the sample from the fishing municipality, which presented a percentage of ash higher than the required 5.96%.

Subsequently, ethanolic extracts of the propolis were obtained in order to synthesize magnetic nanoparticles with the formula  $Fe_2+Fe_3+2O_4$ . 17 samples were synthesized that and characterized by X-ray diffraction (XRD). It was possible to identify the expected crystal phase, except for two samples. Scanning electron microscopy (SEM), semiquantitative analysis of composition by energy dispersive X-rays (EDX) was performed, thus knowing the morphology and composition of the representative samples. Finally, the magnetic characterization was carried out by vibrating sample magnetometry at temperatures between 50 and 300 K.



#### A preliminary characterization of honey from *Butia yatay* palm groves in Entre Ríos, Argentina

Veronica Busch<sup>1</sup>, Lucía Elisabet Brelis<sup>2</sup>, Carolina Elizabeth Genevois<sup>1</sup>, Agustín Sanguinetti<sup>3</sup>, Pilar Buera<sup>4</sup>

<sup>1</sup>Instituto de Ciencia y Tecnología de Alimentos de Entre Ríos, UNER CONICET, Gualeguaychú, Argentina.

<sup>2</sup>Facultad de Bromatología, Universidad Nacional de Entre Ríos, Gualeguaychú, Argentina.

<sup>3</sup>Instituto de biodiversidad y biología experimental y aplicada, Ciudad Autónoma de Buenos Aires, Argentina

<sup>4</sup>Instituto de Tecnología de Alimentos y Procesos Químicos, Ciudad Autónoma de Buenos Aires, Argentina

The organoleptic, nutritional and even pharmacological properties of honey are closely related to its floral and geographical origin. The palm groves of *Butia yatay* constitute a unique natural landscape with abundant resources for bees in the east of the province of Entre Ríos, Argentina. The objective of this work was to characterize honey associated to the palms' blooming regarding its physicochemical, rheological, sensory and melissopalynological properties. The pollen counts showed that in all the samples the sum of the percentages of pollen from *Butia yatay*, *Eryngium horridum* (Apiaceae) and Myrtaceae species represented 90% of the total, alternating the dominance between these three depending on the hive evaluated. All honeys showed good physicochemical characteristics (water content of 16.0-17.6%; conductivity of 0.769-1.318 mS/cm; HMF  $\leq$ 0.1 mg/kg, proline of 471.8-763.6 mg/kg; reducing sugars of 68.1-79.8%; and glucose of 21.2-30.4%) and good bioactivity (by total polyphenols and antiradical activity). Rheologically, they were Newtonian or slightly pseudoplastic (flow index of 0.98-1.03) at 25 °C. Organoleptically, they were highly sweet, with a floral aroma, and they showed medium sized glucose crystals. This work provides information for the characterization and typification of an unstudied honey type from the Palmar de Colón (Entre Ríos, Argentina) protected area, which would allow the consumer to know the origin and special characteristics of this honey, in addition to adding value to local production, and ensuring quality parameters and traceability.

### PP-352

#### "Polyphenols-Rich Extracts of Chilean Propolis: Extraction and Application in edible films"

Daniela Bustos<sup>1</sup>, Patricia Velásquez<sup>2</sup>, Franco Figueroa<sup>3</sup>, Ady Giordano<sup>3</sup>, Gloria Montenegro<sup>4</sup>

<sup>1</sup>Department of Chemical Sciences, Faculty of Exact Sciences, Andres Bello University, Department of Inorganic Chemistry, Faculty of Chemistry and Pharmacy, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>2</sup>Department of Chemical Sciences, Faculty of Exact Sciences, Andres Bello University, Santiago, Chile

<sup>3</sup>Department of Inorganic Chemistry, Faculty of Chemistry and Pharmacy, Pontificia Universidad Católica de Chile, Santiago, Chile

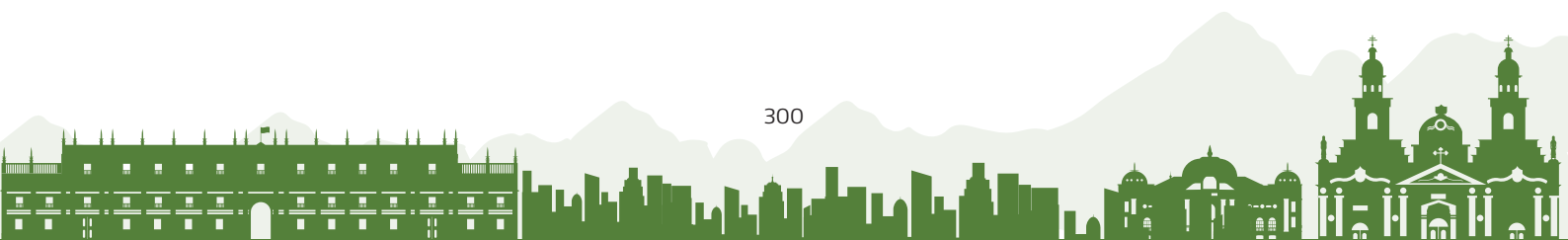
<sup>4</sup>Department of Plant Sciences, Faculty of Agronomy and Forest Engineering; Pontifical Catholic University of Chile, Santiago, Chile.

Propolis is a resinous material collected by honey bees (*Apis mellifera* L.) from various plant sources. Propolis and its extracts have been used in the prevention and treatment of diseases, due to its antioxidant, antibacterial, and antifungal effects, etc. This is attributed to the varied chemical composition of this product, which is made up of resin, waxes, and organic compounds, mainly phenolic compounds, such as flavonoids, phenolic acids, and esters. The main extraction method of these bioactive compounds is based on the use of ethanol and other organic solvents, which, due to their toxicity, limit the use of the extracts in the cosmetic and pharmaceutical industry.

This research aims to evaluate the extraction of bioactive compounds from Chilean propolis, using green solvents assisted with ultrasonic waves, to obtain extracts rich in polyphenols, to later be incorporated into edible films. These solvents were designed and characterized, evaluating their extractive capacity, using the Folin-Ciocalteu method for the quantification of polyphenols. The extracts of propolis obtained presented a polyphenol content between 0.714 and 22.80 mg GAE/g, while their antioxidant capacity was determined using the ABTS method, obtaining values between 21.88 and 61.55  $\mu$ mol ET/g, for the DPPH methodology of 12.17 to 30.48  $\mu$ mol ET/g and for FRAP method from 32.18 to 56.26  $\mu$ mol ET/g. These results indicate a proportionality between the polyphenol content and its antioxidant activity, in addition, the highest values are similar and comparable when using ethanol as a control. This tells us that this methodology is a good alternative for the extraction of bioactive compounds from Chilean propolis. Finally, polyphenols-rich extracts incorporated into edible films transfer their antioxidant characteristics.

Yıldırım, H. K. (2022). Brazilian Archives of Biology and Technology, 65.

Kubiliene, L. (2015). BMC complementary and alternative medicine, 15(1), 1-7.





### PP-353

#### Hydrogen peroxide production on simulated honey induced by different flavonoids in the presence of Cu (II)

Javiera Sanhueza, María Báez, Edwar Fuentes

Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

Honey has positive nutritional effects and beneficial properties including antibacterial activity (ABA). Several factors are associated with ABA, such as acidic pH (3.5-5.0), osmotic pressure, polyphenols (PFs), the peptide defensin-1 and the generation of hydrogen peroxide ( $H_2O_2$ ). PFs of honey would have a major role in the generation of ROS and ABA due to its "pro-oxidant" action modulated by some metallic cation like Cu (II). However, the relationships between these components of honey and ROS are not fully understood. In the present work, the generation of  $H_2O_2$  by autooxidation of the flavones chrysin, and apigenin; the flavonols quercetin, kaempferol, myricetin and rutin; and gallic acid was evaluated in a synthetic honey (as simplified model); in the absence and presence of Cu (II). It was observed that the generation of  $H_2O_2$  by the flavonols was favored by adding Cu (II), possibly due to a mechanism of complexation and subsequent oxidation of these compounds, due to their molecular structure. While the generation of  $H_2O_2$  by the flavones in the presence of Cu (II) was equal to or less than in absence of this cation; which indicates that the autoxidation of flavones as mechanisms of  $H_2O_2$  generation prevails. On the other hand, it was observed that the generation of  $H_2O_2$  by flavonoids in synthetic honeys is additive; while in real honey this action would be synergistic which shows that other components of the honey matrix could be a fundamental role in this regard.

### PP-354

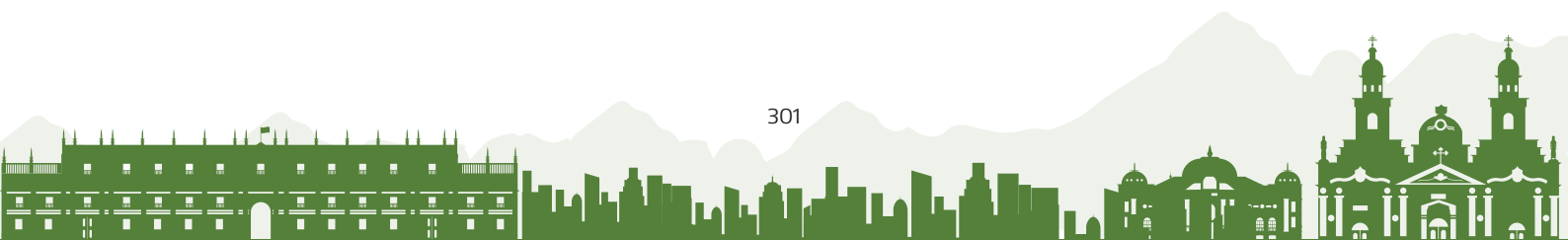
#### Evaluation of the generation of reactive oxygen species and antibacterial activity of honey as a function of its phenolic and mineral composition

Edwar Fuentes<sup>1</sup>, Ximena Faúndez<sup>1</sup>, María Báez<sup>1</sup>, Jessica Martínez<sup>2</sup>, María Zúñiga<sup>1</sup>, Jeannette Espinoza<sup>1</sup>

<sup>1</sup>Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

<sup>2</sup>Centro de Medicina Regenerativa, Facultad de Medicina, Clínica Alemana-Universidad del Desarrollo, Santiago, Chile.

Honey is a common food in the human diet, exhibiting positive nutritional effects and beneficial properties including antibacterial activity (ABA). Several factors are associated with ABA, such as acidic pH (3.5-5.0), osmotic pressure, polyphenols (PFs), the peptide defensin-1 and the generation of reactive oxygen species (ROS), such as  $H_2O_2$  and the hydroxyl radical. PFs of honey would have a major role in the generation of ROS and ABA due to its "pro-oxidant" action modulated by some metallic cation. However, the relationships between these components of honey, ROS, and its ABA are not fully understood. In this work, the contents of PFs,  $H_2O_2$ , OH radical, Cu, Fe, Mn, Zn, and ABA against *S. epidermidis* and *P. aeruginosa* were determined in honeys from central Chile. Then, their relationships were evaluated through partial least square regression (PLS). The frequency of appearance of phenolic acids in honey was gallic > p-coumaric > caffeic > ferulic > syringic > chlorogenic (average 0.4-4.1  $\mu\text{g/g}$ ); while for flavonoids was quercetin > chrysin > kaempferol > apigenin > myricetin = rutin (average 0.3-1.5  $\mu\text{g/g}$ ). The metal concentration was Fe > Cu > Zn > Mn (average 2.5-6.1  $\mu\text{g/g}$ ). All honeys showed accumulation of  $H_2O_2$  (1.0-35  $\mu\text{g/g}$ ) and OH radical formation. The honeys had lower MIC value against *S. epidermidis* than *P. aeruginosa* (average 14 and 18% v/v, respectively). The PLS showed that phenolic acids (particularly gallic acid, p-coumaric acid, chrysin, and kaempferol); Fe, and Mn have a pro-oxidant effect stimulating the generation of ROS. While quercetin and Cu have a marginal antioxidant effect. The PFs showed an effect on the antibacterial activity of honey against *S. epidermidis* and *P. aeruginosa* as a group rather than individually. The  $H_2O_2$  mainly determines the antibacterial activity against *S. epidermidis*. On the contrary, *P. aeruginosa* is less susceptible to ROS than *S. epidermidis*.



### PP-355

#### Development of an analytical method for the determination of defensin-1 in honey based in liquid chromatography

Pablo Correa<sup>1</sup>, Iván Opazo<sup>1</sup>, Mauricio Báez<sup>2</sup>, María Báez<sup>1</sup>, Edwar Fuentes<sup>1</sup>

<sup>1</sup>Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

<sup>2</sup>Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

Since 2006 the honeybee (*A. mellifera*) has experienced a manifest decline in its population caused by a phenomenon called Colony Collapse Disorder, which has been associated with various stressors such as pesticides, parasites and pathogens. To cope with these stressors *A. mellifera* has developed social immunity mechanisms among which is the secretion of the antibacterial peptide (ABP) defensin-1 to the hive food: royal jelly and honey. However, there are limited reports on the determination of defensin-1 in honey. Since this ABP would give to honey antibacterial activity represents a good biomarker to characterize and verify the quality of this product. In this work, we present the development of a method for the separation, detection and quantification of defensin-1 in honey based on C18 solid phase extraction (SPE) and determination by ion-pair reversed-phase high performance liquid chromatography (IP-RP-HPLC), coupled to a fluorescence detector. First, recombinant defensin-1 (used as a standard) was purified by immobilized metal affinity chromatography (IMAC), followed by size exclusion chromatography. The identity of the purified protein was verified by liquid chromatography with tandem mass spectrometry (LC-MS/MS); with 100% fit of the amino acid sequence and a weight (theoretical weight 5.525 Da). Gradient elution (water trifluoroacetic acid 0.05% / acetonitrile trifluoroacetic acid 0.05%) permitted the separation and detection of defensin-1; with linear response between 0.1 and 2.0  $\mu\text{M}$  ( $R^2 = 0,99$ ) and LOQ = 0.1  $\mu\text{M}$ . Defensin-1 was determined in honey samples analyzed from the central Chile, obtaining a mean value 2  $\mu\text{g/g}$  of honey. Thus, the method based on SPE-C18 associated to HPLC-FD allowed the quantification of defensin-1 in Chilean honeys.

### PP-356

#### Chemometrics and Physicochemical properties of Chilean honeys produced from native species in the Ranco Lake Zone (Los Rios Region)

Enrique Mejías

Centro de Tecnologías Nucleares en Ecosistemas Vulnerables CTNEV. División de Investigaciones y Aplicaciones Nucleares DIAN. Comisión Chilena de Energía Nuclear CCHEN

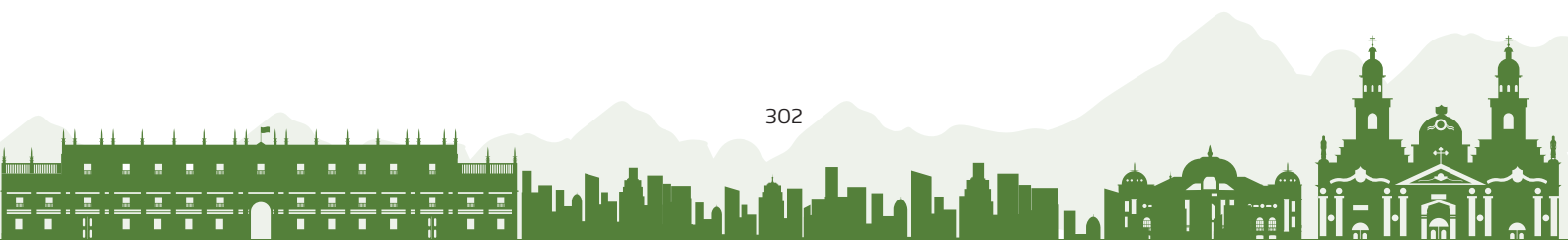
Chile has a great variety of native endemic melliferous flora distributed across the country from north to south. Likewise, the quality of Chilean honeys is recognized abroad mainly for good taste and natural biological properties due to their botanical origin.

The Chilean production of honeys occurs in several regions, however in the southern there are several native rainforests with natural conditions protected from mankind. Thus, honeys obtained from those zones are very valued, but they still remain with limited analytical characterization.

In this study, 7 apiaries located in the proximity of Ranco Lake zone (Los Rios Región) were selected and Honeys of each place were collected in three consecutive harvesting seasons at the end of summer (Total honey samples: 21). Samples were analyzed to Botanical origin, % of humidity, pH and content of Ashes, Protein, Total Carbohydrate, Total Phenolic compounds. Also, a ratio of Antioxidant activity/Phenolic compounds content (AA/PCC) was calculated from the obtained data.

The results suggest a strong influence of two native species in the antioxidant activity: *Caldcluvia paniculata* and *Weinmannia trichosperma*, but the most remarkable findings is related to the combination of those species in the final botanical composition of honeys.

Data and correlations among parameters are shown by considering the distribution of apiaries in the zone.



### PP-357

#### Hydoxymethylfurfural content in Honeys from the rural areas of Azad Kashmir Pakistan

Ayesha Mushtaq<sup>1</sup>, Dr. Sofia Khalid<sup>2</sup>, Mehwish Jamil Noor<sup>1</sup>

<sup>1</sup>Environmental sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan

<sup>2</sup>Environmental sciences Department Allama Iqbal Open University, Rawalpindi, Pakistan

Honey bee keeping is emerging in rural areas of Azad Kashmir Pakistan. Honey is marketed from wild, household bee keeping and Farm beekeeping at local and national level. Hydroxymethylfurfural (HMF) content is the degradation of fructose in extreme thermal conditions for a longer period. It is considered as freshness indicator. Rural areas of Azad Kashmir with diverse flora and favorable environmental conditions are famous for honey beekeeping. A total of sixty multifloral honey samples were collected from local beekeepers in three divisions of Azad Kashmir Pakistan. HMF content was determined spectrophotometrically at 284nm and 336nm in accordance with white's method by International Honey Commission (IHC). It was found that twenty nine samples exceeded the Codex Alimentarius limit for HMF content i.e. 40 mg/kg. This increase in HMF is due to the storage at room temperature for longer period after processing at high temperature in rural mountainous setting. HMF content can be prevented by avoiding thermal treatment and storage for longer period.

### PP-358

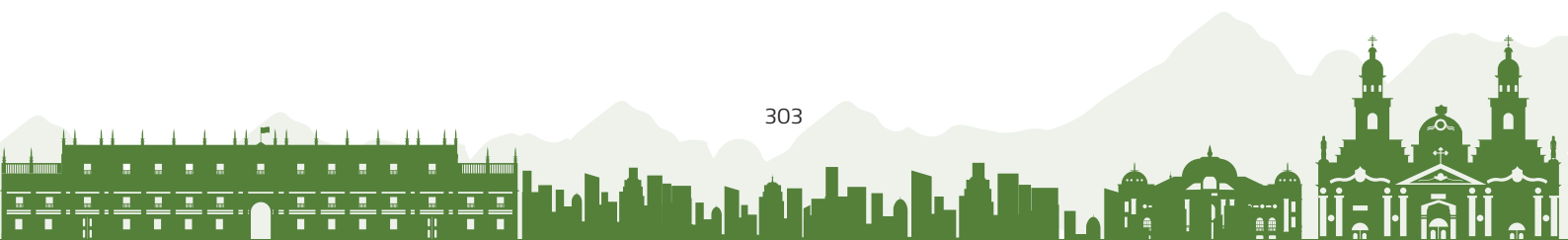
#### Monitoring of organic contaminants in honey and soils in southern Chile

Tatiana Garrido<sup>1</sup>, Enrique Mejias<sup>2</sup>, Jorge Mendoza<sup>1</sup>, Patricio Lagos<sup>1</sup>

<sup>1</sup>Department of Inorganic and Analytical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile

<sup>2</sup>Chilean Nuclear Energy Commission - CCHEN, Chile

The composition of honey depends on its botanical origin and physicochemical characteristics of the soil near the hives. Soils may contain organic and inorganic pollutants depending on the anthropogenic activities in them, being transported to the hives through the interaction of bees with water, plants and soil. Identifying the geographic origin of honey can help consumers and producers to prevent bees from being exposed to contaminants present in feeding areas or to select the location of the hive with the least risk of contamination. The objective of this study was to correlate the contaminants present in soils near hives with those present in honey. A sampling of soils and honey from Temuco and Valdivia, southern Chile, was carried out. Soil samples were characterized by standardized methods (pH, organic carbon content, metal content, etc.). For the extraction of organic contaminants from the soil, a solid-liquid extraction with ultrasound was performed. Through the techniques of Gas Chromatography with electronic microcapture detector (GC- $\mu$ ECD) and direct sample analysis - time of flight - mass spectrometry (DSA-TOF-MS) it was possible to quantify and confirm the presence of organic contaminants both in honey as in soils. The results of the soil characterization showed a pH that fluctuated between 4.56 and 6.57, with a high organic carbon content. The m/z spectra obtained using the DSA-TOF-MS technique of aqueous extracts revealed the presence of organic contaminants, such as the organophosphorus pesticide chlorpyrifos, with a half-life greater than 60 days in soils and honeys of at least three years. sampling sectors. Another of the contaminants identified by DSA-TOF-MS is the fungicide fenhexamide, with a half-life of 1 day under aerobic conditions, which was only found in honey extracts. It is concluded that the DSA-TOF-MS technique allows determining the presence of contaminants in honey from the sampled sectors, and that the extraction method described is a simple and low-cost technique, which allows the quantification of organic contaminants. Finally, it was possible to verify that there is a transfer of organic pollutants between soil, bee, hive and honey.



### PP-359

#### Study of montes del queguay honeys: a model to work in the characterization and valorization of specific valuable honeys

Selva Cora Bessio<sup>1</sup>, Gabriela Tamaño<sup>1</sup>, Gloria Daners<sup>3</sup>, Eduardo Dellacassa<sup>2</sup>

<sup>1</sup>Laboratorio de Tecnología de la Miel y Productos Apícolas - ApiUTEC - LAA-ITRSO-UTEC Universidad Tecnológica, Paysandú, Uruguay

<sup>2</sup>Laboratorio de Biotecnología de Aromas-DQO-CYTAL, Facultad de Química UdelAR, Montevideo, Uruguay

<sup>3</sup>Instituto de Ciencias Geológicas, Facultad de Ciencias UdelAR, Montevideo, Uruguay

The "Montes de Queguay" Protected Area is located in the Uruguay river bank, an extensive region with abundant and diverse native flora represented in different natural environments. In this ecosystem, beekeepers produce honeys with unique characteristics. Our research group seeks to develop specific knowledge to characterize these honeys, aiming at their differentiation applicable as an added value for them in the market. For this purpose, the analytical methodology defined implies not only the melissopalynological analysis of honeys, but also their physicochemical characterization and aroma profile study (GC-MS).

Here we present the results obtained using 17 honey samples from georeferenced apiaries in the summer 21/22 harvest. By combining both types of analysis (chemical and pollinic), it was possible to obtain a more comprehensive understanding of the properties and characteristics of this region honeys.

Honeys volatile profiles refers to the aroma and flavor compounds present in honey. These compounds can vary depending on several factors, including nectar sources, geographic location, and processing methods used, where polinic analysis allows identification and quantification of honeys floral sources. In fact, pollen analysis is the common method used to determine honeys botanical origin. However, there could be several reasons why honeys volatile profiles may not be related to the pollinic analysis. Moreover, honeys volatile profile can change over time due to factors such as storage conditions, temperature, and exposure to light and air, while the pollinic analysis remains constant.

A total of 84 pollen types were identified (32 species level, 25 genus, 21 family, 6 remain undetermined). In more than 60 % of the samples, native trees *Scutia buxifolia*, *Blepharocalyx salicifolius* and *Terminalia australis* represented more than 74% of the spectrum. More than 80 volatile compounds were identified, where terpene compounds accounted for almost 40% of the total composition. Through PCA statistical analysis, no direct associations were observed between melissopalynological analysis and volatile profiles in the samples studied. The results seems to demonstrate that, while the pollinic analysis provides important information about honeys botanical origin, it may not necessary be always directly related to honeys aroma and flavor profile, at least for the region under study.

### PP-360

#### Professionalization of Apiculture in Brazil using the e-Learning System, Distance Education

Lidia Maria Ruv Carelli Barreto<sup>1</sup>, Denise de Lima Belisario<sup>2</sup>, João Carlos Nordi<sup>3</sup>, Francisco Giovanni Laércio<sup>4</sup>, Ricardo de Oliveira Orsi<sup>5</sup>

<sup>1</sup>Lidia Maria Ruv Carelli Barreto

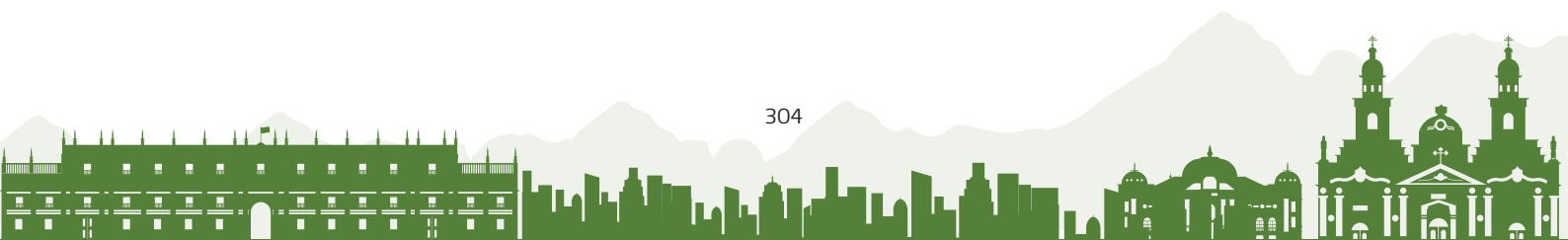
<sup>2</sup>Denise de Lima Belisario

<sup>3</sup>João Carlos Nordi

<sup>4</sup>Francisco Giovanni Souza Laércio

<sup>5</sup>Ricardo de Oliveira Orsi

Brazil, a country with continental dimensions, presents 8.514.876 Km<sup>2</sup> of territorial extension, so, also demands educational strategies that reach a larger number of its population. In order to improve the "learning on-the-job" system, where only 15% of knowledge comes from formal learning, UNITAU (University of Taubaté), attentive to the demands of the area at national level, has undertaken in the training of the beekeeper and meliponicultor by the Distance Education System (in Brazil - EAD), being today the only institution with two higher education and postgraduate courses specialized in the modality of Apiculture and Meliponiculture and which has successfully been assisting the professional revolution in the country. It is estimated that in Brazil there are about 550.000 beekeepers, mostly demanding professional training. The course aimed at training the leaders in several Brazilian regions that remains in full operation until these days, attracting even leaderships from other Latin American Countries. The Courses are recognized by the Ministry of Education (in Brazil - MEC), with an excellent score, they are in expansion and already implemented in Bahia State and about to be implemented in several states of Brazil. It is believed that in a Country as Brazil, this model is one of the most efficient system, capable of reaching and mobilizing many, making the necessary changes towards professionalization of Apiculture and Brazilian Meliponiculture. In these 18 years, the course has served more than 2,000 students who have multiplied the knowledge in the most remotes regions of this Country.



### PP-361

## Stable Isotopic Techniques for the determination of authenticity in honey: implementation and validation of the analytical method in Chilean honeys

Enrique Mejías<sup>1</sup>, Simon Kelly<sup>2</sup>, Nuri Gras<sup>3</sup>

<sup>1</sup>Centro de Tecnologías Nucleares en Ecosistemas Vulnerables - División de Investigación y Aplicaciones Nucleares - Comisión Chilena de Energía Nuclear

<sup>2</sup>Food Safety and Control Laboratory - IAEA Laboratories Seibersdorf

<sup>3</sup>Departamento de Ciencia de los Alimentos y Tecnología Química - Facultad de Ciencias Químicas y Farmacéuticas - Universidad de Chile

In recent years, honey production has been optimized and the characterization of honey and its natural attributes has allowed its valorization and differentiation. However, at the same time, the counterfeiting of bee products has been increasing, causing great damage to beekeeping and thus generating significant economic losses for beekeepers. Also, the health of people who consume adulterated products is exposed to various risks since fake products do not have a defined origin and their composition may contain residues or undesirable elements.

In Chile, fake honeys occur and is commercialized in non-formal markets and, therefore, there is concern from the beekeeping production sector to address this problem with scientific tools and methodologies allowing the certification of the authenticity of Chilean honeys. In fact, all of them must also comply with strict mandatory regulations in the destination countries where they are exported.

This work presents an overview of the methodology for the processing and subsequent analysis of the <sup>13</sup>C/<sup>12</sup>C isotopic ratios obtained for 20 Chilean honeys of different botanical origins analyzed by IRMS - EA. Likewise, the results were correlated with natural biological attributes of the studied samples, with the purpose of building a database on authenticity, geographical origin, and index of bioactive molecules in Chilean honeys.

A total of 84 pollen types were identified (32 species level, 25 genus, 21 family, 6 remain undetermined). In more than 60 % of the samples, native trees *Scutia buxifolia*, *Blepharocalyx salicifolius* and *Terminalia australis* represented more than 74% of the spectrum. More than 80 volatile compounds were identified, where terpene compounds accounted for almost 40% of the total composition. Through PCA statistical analysis, no direct associations were observed between melissopalynological analysis and volatile profiles in the samples studied. The results seem to demonstrate that, while the pollinic analysis provides important information about honeys botanical origin, it may not necessarily be always directly related to honeys aroma and flavor profile, at least for the region under study.

### PP-362

## The concentration of melittin and apamin in the apitoxin vary depending on whether external or internal traps are used

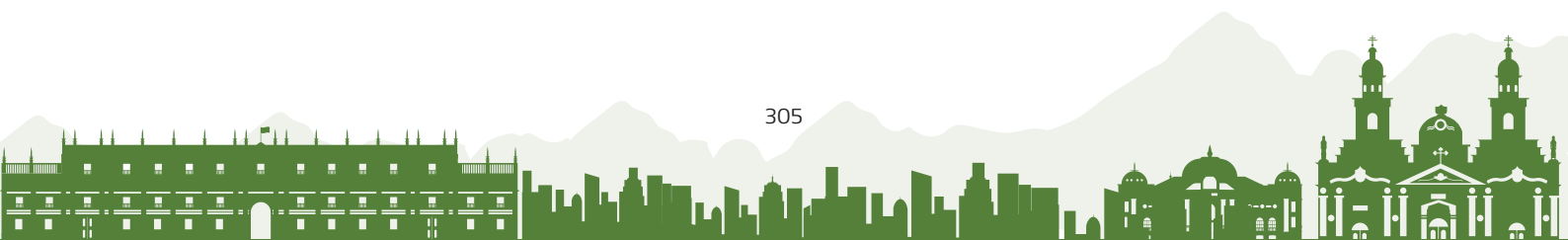
Agustín Pérez Graña<sup>1</sup>, Valentina Rodríguez Batista<sup>2</sup>, José Anzola<sup>2</sup>, Zonia Martínez<sup>3</sup>, Pablo Andrés Juri<sup>2</sup>, Enrique Nogueira<sup>2</sup>, Catherine Fagúndez<sup>2</sup>

<sup>1</sup>Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay

<sup>2</sup>Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay

<sup>3</sup>Instituto de Farmacia y Alimentos, Universidad de la Habana, Habana, Cuba

Commercial apitoxin is obtained using an electrical current emitting equipment connected to the traps that have a metallic grid with a glass attached, so that when the bees land on the circuit it closes it and receives a small electric shock that triggers a sting. The apitoxine is deposited in the glass without killing the bee. Apitoxine is composed by tens of bioactive substances that start to synthesize since the adult bee emerges till it completes its venom sack around three weeks later. The traps can be located in the inside or outside of the hive, the bees that will access one or the other are going to be younger or more matured due to temporal polyethism. The goal was to evaluate if the place where the hive is placed affects the composition of the apitoxin. The experiment was performed in the Experimental Field of the Faculty of Veterinary using 20 hives, all with an estimated population of over 40,000 adult bees. They were divided into Group IT (internal traps) and Group ET (external traps), the extraction was performed in the afternoon for 30 minutes. The glasses corresponding to each colony were identified and the HPLC percentage of melittin and apamin of the harvested apitoxin was quantified in each hive in the experiment. The results were evaluated using a variance analysis for a completely randomized design and significant differences in melittin were found, with IT 72.63%, ET 62.37% (p-value 0.036) and in apamine, IT 1.55%, ET 1.47% (p-value 0.005). These differences are possibly caused by a different speed of the synthesis of the different components of the apitoxin, so that the composition would vary according to the age of the bees and therefore the composition of the harvested apitoxin is going to vary according to whether the traps are visited by younger bees (IT) or mature bees (ET) which opens the possibility to direct the production of the apitoxin with a higher percentage of melittin using internal traps.





### PP-363

### Ultrasound-assisted extraction of polyphenols from Chilean bee pollen for its incorporation into edible films

Franco Figueroa<sup>1</sup>, Daniela Bustos<sup>1</sup>, Ady Giordano<sup>2</sup>, Gloria Montenegro<sup>3</sup>, Patricia Velásquez<sup>4</sup>

<sup>1</sup>Department of Inorganic Chemistry, Pontifical Catholic University of Chile, Santiago, Chile, Department of Chemical Sciences, Andres Bello University, Santiago, Chile

<sup>2</sup>Department of Inorganic Chemistry, Pontifical Catholic University of Chile, Santiago, Chile

<sup>3</sup>Department of Plant Sciences, Pontifical Catholic University of Chile, Santiago, Chile

<sup>4</sup>Department of Chemical Sciences, Andres Bello University, Santiago, Chile

Bee pollen is a granular material produced by the agglutination of floral pollen, nectar of honey and salivary substances from honeybees (*Apis mellifera* L.). Its constituents are proteins, carbohydrates, lipids, fiber and vitamins, which give it great nutritional value. Besides them, bioactive compounds in bee pollen, such as polyphenols and carotenoids, can be extracted and added into edible films, to enhance their functional properties for food packaging. These compounds can be extracted with ultrasounds, an eco-friendly technique due to the short time required and less energy consumption.

The aim of this research is the solid-liquid extraction of polyphenols from Chilean bee pollen applied with ultrasounds and the incorporation of these extracts into edible films. The optimal parameters of the extraction of polyphenols were obtained using response surface methodology with three levels: ultrasound amplitude, time and solvent concentration. The quantification of polyphenols in the optimized extracts were evaluated using the Folin-Ciocalteu method, and the mean results were  $120.5 \pm 5.4$  mgGAE/100g. The antioxidant capacity was evaluated by %DPPH inhibition, obtaining a mean value of  $74.9 \pm 0.9$  %. These results indicate that the extracts obtained have a comparable antioxidant capacity to ethanol, which was used as a control. Physicochemical properties of the synthesized edible films were evaluated, such as, thickness, CIELab color, angle contact, UV-screening and antioxidant capacity. The addition of the extracts into the edible films modified the antioxidant capacity, thickness and UV protection but did not change color, transparency and contact angle of the films.

### PP-364

### NAPs: participatory learning groups as social innovation on beekeeping

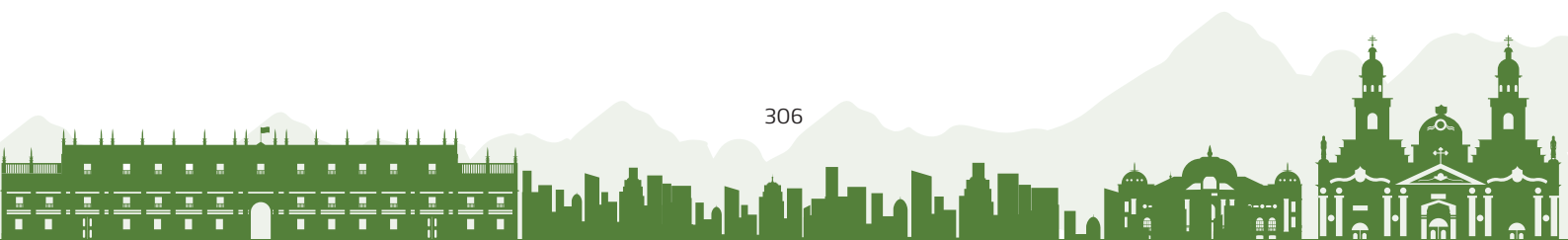
Francisco Carvallo, Marta Albornoz, Armando Alfaro Tapia

Centro Regional de Investigación e Innovación para la Sostenibilidad de la Agricultura y los Territorios Rurales, Centro Ceres, Pontificia Universidad Católica de Valparaíso, Quillota, Chile.

Centro Ceres is enforcing an extension and transfer methodology for beekeeping, called Núcleos de Aprendizaje Participativo (NAPs) or Participatory Learning Groups. This methodology is based on the sharing of knowledge among beekeepers, technicians, professional members from Local Development Program (PRODESAL) and the scientific community. Its objective is to generate interactivity among entities and collaboration networks to promote innovation processes among beekeepers, strengthening learning and through the co-construction of knowledge. The NAPs are constituted as physical areas that serve as demonstration modules and meeting points for workshops. Through the implementation of this methodology, knowledge is shared horizontally, presenting the professional members in charge of each workshop as someone close and who greatly values the beekeeper's experience, generating a meeting point among the participants who can share their knowledge.

At present, two NAPs have been created that host beekeepers from Olmué, Quillota, Catemu, Santa María, Panquehue and Llay Llay communes in the Valparaíso Region, Chile. It has been very important to have the support and motivation of PRODESAL professionals and technicians, as they are a key element in the process of implementing the NAPs as a tool for social innovation in beekeeping.

Our results highlight the importance of democratizing and validating the knowledge that beekeepers bring to the group, with respect to the agreements reached among the participants, as well as creating a pleasant and relaxed environment. This social innovation tool has allowed the participants to find an opportunity for thoughtful learning about their activities based on their own experiences to develop trust, stimulating the transition towards sustainable beekeeping



## The Journal of Apicultural Research welcomes the publication of research findings from around the globe

**Melanie Parejo<sup>1</sup>, Adriana M Alippi<sup>2</sup>, Otilia Bobis<sup>3</sup>, Robert Broschneider<sup>4</sup>, Vanessa Corby Harris<sup>5</sup>, Maria Dimou<sup>6</sup>, Orawan Duangphakdee<sup>7</sup>, Anna Gajda<sup>8</sup>, Dora Henriques<sup>9</sup>, Irfan Kandemir<sup>10</sup>, Robert Pickard<sup>11</sup>, Juliana Rangel<sup>12</sup>, Stuart Roberts<sup>13</sup>, Victoria Soroker<sup>14</sup>, Jevrosima Stevanovic<sup>15</sup>, Maria Bouga<sup>16</sup>**

<sup>1</sup>Applied Genomics and Bioinformatics, University of the Basque Country (UPV/EHU), Leioa, Spain

<sup>2</sup>Cátedra de Fitopatología, Facultad de Agronomía, Universidad Nacional de La Plata, La Plata, Argentina

<sup>3</sup>Department of Apiculture and Sericulture, Faculty of Animal Science and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

<sup>4</sup>Institute of Biology, University of Graz, Austria

<sup>5</sup>USDA's Carl Hayden Bee Research Center, Tucson, Arizona, USA

<sup>6</sup>Laboratory of Apiculture and Sericulture (School of Agriculture), Aristotle University, Thessaloniki, Greece

<sup>7</sup>King Mongkut's University of Technology Thonburi, Ratchaburi Campus, Ratchaburi, Thailand

<sup>8</sup>Laboratory of Bee Diseases (Institute of Veterinary Medicine), Warsaw University of Life Sciences, Warsaw, Poland

<sup>9</sup>Mountain Research Centre (CIMO), Polytechnic Institute of Bragança, Bragança, Portugal

<sup>10</sup>Department of Biology, Ankara University, Ankara, Turkey

<sup>11</sup>CoRWM, Department of Energy and Climate Change, United Kingdom

<sup>12</sup>Department of Entomology, Texas A&M University, College Station, Texas, USA

<sup>13</sup>Freelance Entomologist and Ecological Consultant, Salisbury, England

<sup>14</sup>Department of Entomology, Chemistry and Nematology, Institute of Plant protection, Agricultural Research Organization, The Volcani Center, Rishon LeZion, Israel

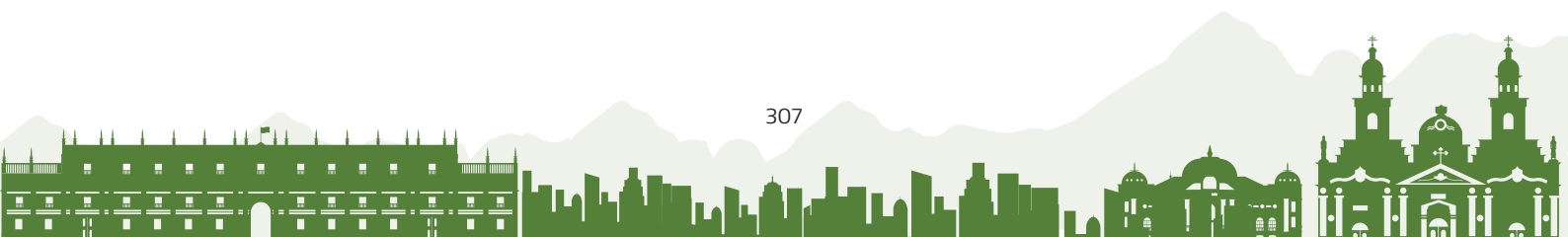
<sup>15</sup>Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

<sup>16</sup>Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, Athens, Greece

The Journal of Apicultural Research (JAR) is a peer-reviewed, scientific journal dedicated to examining and publishing the latest research on bees from around the world. JAR publishes many different types of articles to reach different international audiences, from career scientists to students and well-informed beekeepers. These comprise original, theoretical, and experimental research papers, as well as authoritative notes, comments, and reviews on scientific aspects of all types of bees (superfamily Apoidea). As of 2021, JAR has an Impact Factor of 2.407 and is ranked 33rd out of 100 in the Entomology category (© InCites Journal Citation Reports®, Clarivate Analytics, 2022). Five regular issues are published per year and special issues are added when timely topics arise, the latest being a special issue on stingless bees (2022) and review papers (2023).

In the last decade, COLOSS BEEBOOK chapters are published in JAR. These open-access chapters are a collection of the Standard Methods used in honey bee research, including the study of parasites, pests, and hive products. They are a primary reference resource for bee researchers across the globe and facilitate new projects that might not otherwise be undertaken by laboratories that are new to apidology (236,516 downloads - Taylor & Francis 3,028 citations - Web of Science, 2022).

The Journal of Apicultural Research was founded by the International Bee Research Association (IBRA) in 1962. The very first issue included a Note from the first Editors, Dr. Eva Crane & Dr. James Simpson, who introduced JAR as a new opportunity for publication: "The journal will cover all aspects of bees, Apis and non-Apis, and substances used or produced by them, their pollinating activities, and organisms causing diseases or injuries to them." Since the first issue, this legacy has been maintained in more than 2,800 scientific articles, co-authored by some 1,900 researchers, published so far in JAR, making our journal a key forum for the international exchange of scientific data in apidology. We encourage colleagues from around the globe to continue to participate in sharing their research with the scientific community by publishing in JAR.



### PP-366

#### Some current trends in the residue situation derived from recent honey analyses

Hartmut Wischmann, Hauke Zinow, Peter Tebbe, Harald König, Klaus Beckmann

Intertek Food Services GmbH, Bremen, Germany

In recent years, the residue analyses of honeys or bee products carried out by Intertek Food Services in Bremen have shown a number of shifts from previously frequently positive analysed residue groups or contaminants to new substance classes. While antibiotic groups lost importance, at least in some regions of origin, and in particular so-called prohibited substances were detected significantly less frequently, new active substances, often insecticides, moved into focus: The inclusion of matrine and oxymatrine in the scope of pesticides analysed increased the proportion of investigated honeys that did not comply with the legal EU regulation for pesticide residues (Regulation (EC) No. 396/2005), as corresponding maximum residue levels (MRLs) were exceeded. Matrine and oxymatrine are alkaloids extracted from plants of the genus *Sophora*. In several Asian countries (e.g. Bangladesh, China, Mongolia, Myanmar, Vietnam) these substances are also used as pesticides.

Another point is to turn to active substances with comparable activity, in order to avoid other compounds that are considered very critical. The controversial neonicotinoid thiacloprid is increasingly being replaced by the insecticide acetamiprid, also a neonicotinoid compound. Other compounds have come into focus following adapted legislation. The maximum residue level of 0.05 mg/kg introduced for chlorate and the frequently applied orientation value at the same level for perchlorate led to further investigations, as a result of which the situation with regard to positive findings could be illuminated for the first time.

Corresponding results are presented with evaluations of the last investigation periods.

### PP-367

#### Development of a Mobile Application for Beekeeping Production Control and Registration in the Cluster Apícola Cuenca del Río Salado

Emanuel Caluva<sup>1</sup>, Emilio Figini<sup>2</sup>, Matias Toledo<sup>3</sup>, Alfonso Lorenzo<sup>4</sup>, Pablo Joaquin Moja<sup>5</sup>

<sup>1</sup>EEA Concepción del Uruguay, INTA, Concepción del Uruguay, Argentina

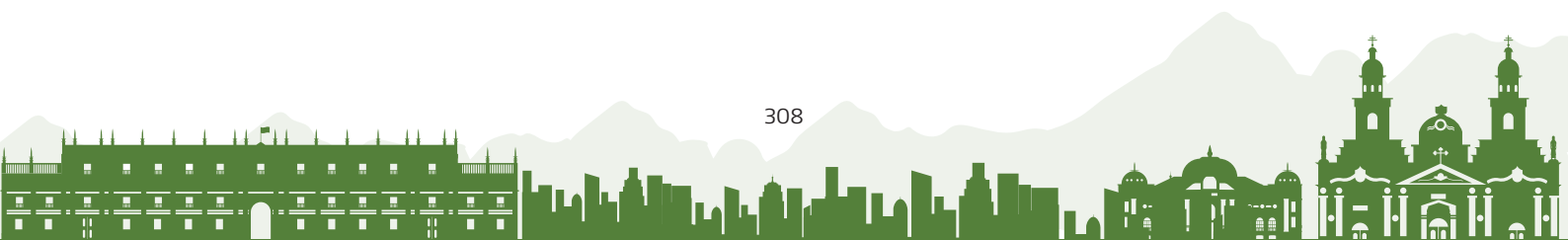
<sup>2</sup>Dto de Producción Animal, FCV. UNICEN, Tandil, Argentina

<sup>3</sup>Asesor Privado, Tandil, Argentina

<sup>4</sup>Programa Cambio Rural, Secretaria de Agricultura Ganadería y Pesca, Tandil, Argentina

<sup>5</sup>EEA Cuenca del Salado, INTA. Rauch, Buenos Aires, Argentina.

As part of a plan to strengthen and simplify the audit system being carried out with beekeepers belonging to the Clúster Apícola Cuenca del Río Salado, the development of a computerized system for registration and production control was proposed. Critical points, types of records, and requirements analysis were identified to define the characteristics of the computerized registration and production control system. As a result of this process, it was concluded that a mobile application would be the best option. Two user profiles were considered: beekeepers and beekeeping technicians, each with different types of access and functions. Android Studio IDE was used to develop the mobile application with a connection to an online database. The application includes geolocation functions for beehives, creation of different types of records based on the Field Sheet recommended by INTA, the ability to create records without an internet connection, a reports section providing quick data based on previously loaded records. Additionally, users can export or share their records for further analysis in other software tools. The result is a user-friendly application that generates a digital record of the movements in the beehive, facilitating the auditing process and contributing to the assurance and control of quality.



### PP-368

#### Stability evaluation at different storage temperatures of high honey content powders

Roy Rivero<sup>1</sup>, Veronica Maria Busch<sup>1</sup>, Mauro Melchiori<sup>2</sup>, Natalia Sosa<sup>1</sup>, Diego Alberto Archaina<sup>1</sup>, Bertha Mabel Baldi Coronel<sup>1</sup>

<sup>1</sup>Instituto de Ciencia y Tecnología de Alimentos de Entre Ríos, ICTAER UNER CONICET, Gualeguaychú, Argentina

<sup>2</sup>Facultad de Bromatología, Universidad Nacional de Entre Ríos, Gualeguaychú, Argentina.

For the last decades the food industry has searched healthier ingredients and sweeteners, and honey is a good candidate to response. However, honey high viscosity and sugar content make it difficult to apply in many new products. By freeze-drying it is possible to preserve the bioactive properties obtaining an easy dispersible powder. The 70% honey powder (using a combination of Arabic gum and WPC to stabilize the sugars) has possible applications in healthy foods and special diets, since in previous studies it was shown to have good both hydration properties and sensory acceptance. In this work, the influence of temperature during storage was evaluated and the powder shelf life was defined. During 12 months, samples were stored at different temperatures (20, 27 and 35°C) to analyse the antioxidant capacity, solubility (S), colour variation (CV) and caking (C). An inverse relationship between S and C was observed, since the former decreases as compaction increases. In addition, the perception of lightness also changes inversely in relation to the increase in C, which is accompanied by an increase in other colour parameters (maybe due to the appearance of brown pigments that cause the increase in CV). According to the Arrhenius equation, the CV has been determined as the limiting parameter, and a storage time of 9 months at room temperature (25°C) has been established to avoid a noticeable colour difference. Concluding, it was determined that the brown products were the limitations in storage time, so high temperatures must be avoid in order to extend their useful life and thus be able to apply them in the formulation of different foods.

### PP-369

#### Characterization of Lotus honey obtained in the lower Delta of Parana River and coastal fringe, Argentina

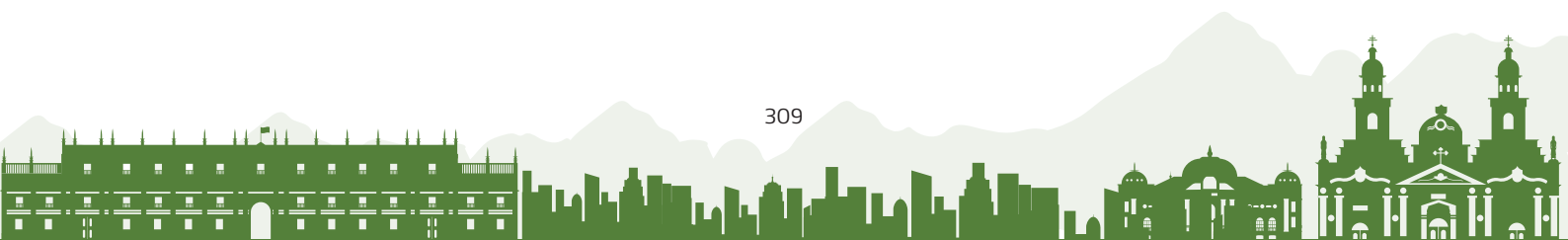
Valeria Carolina López<sup>1</sup>, Laura Beatriz Gurini<sup>1</sup>, María Cristina Ciappini<sup>2</sup>, María Alejandra Palacio<sup>3</sup>

<sup>1</sup>National Institute of Agricultural Technology, Agricultural Experimental Station (EEA) Delta of Paraná, Campana, Buenos Aires, Argentina.

<sup>2</sup>Center for research and development in food technologies, Rosario Regional College, National Technological University, Rosario, Santa Fé, Argentina.

<sup>3</sup>National Institute of Agricultural Technology, Agricultural Experimental Station (EEA) Balcarce, Balcarce, Buenos Aires, Argentina.

The Paraná River Delta has a high floristic diversity, which allows to get different multifloral and monofloral honeys, "Lotus Honey" among then. In order to get the objective of characterize it to add value, 23 samples obtained in the Lower Delta of the Paraná River and the coastal Strip were analyzed, in the years 2006, 2008, 2010, 2013, 2018 and 2019. Pollen analyzes were carried out, using the Method of Wodehouse, Physical-chemical (color, humidity, F/G ratio, electrical conductivity, acidity, HMF and pH), using AOAC and IRAM methods, and sensory (visual, olfactory, smell, taste and texture evaluation), by means of a panel of 7 trained panelists. Honeys with a content of 45 % or more of pollen of this genus were considered as Lotus monofloral. The averages found for the F-Q parameters were: Color 28 to 60 mm Pfund; Humidity: 17 to 19%; F/G ratio 1.26 to 1.60; Conductivity: 395 to 797 mS/cm; Free acidity: 22 to 29; HMF: 0.7 to 15.mg/kg; pH: 3.47 to 3.73. The sensory analyzes showed honey with fine crystals, a light amber color, with yellow, occasionally greenish notes; Weak to moderate odor, not very persistent, from the families: subtle floral, fruity (transformed fruits, tropical fruits), dry vegetable, warm (vanilla), spicy aromatic, chemical (camphorate), rarely animal (protein). Tastes: moderate sweetness, occasionally with an acid note. Among the trigeminal sensations were identified: refreshing, astringent and rarely slightly spicy. The aroma corresponds to the subtle floral, fruity, dry vegetable, aromatic, warm (vanilla, caramel) families. The texture is unctuous, creamy, occasionally gritty. The persistence is moderate, without residual taste. The % of Lotus pollen of the coastal strip honeys are slightly higher than those present in island honeys (median: 75.8 and 72.8 respectively). The present main accompanying pollen types (in % greater than 3) corresponded to Ammi sp., Amorpha fruticosa, Type Baccharis, Carduus sp., Echium plantagineum, Eucalyptus sp., Ligustrum sp., Salix sp., Gleditsia triacanthos, Trifolium repens, Ludwigia sp., Echinodorus grandiflorus and Sagittaria montevidensis, the last three present only in island honeys. These characteristics make it possible to differentiate Lotus honey from other honeys obtained in the same region.



### PP-370

### Determination of antifungal activity and botanical origin of Chilean propolis extracts obtained in 2023

Jorge Perez Veloz, Cabeza Muñoz Felipe

Universidad San Sebastián

Propolis is a beekeeping product that has a high polyphenol content which determines its biological properties. The objective of this study was to quantify the total polyphenols contained in an ethanolic extract of Propolis (EEP), obtained from samples from three regions of Chile (Valdivia- PCP1, Talca- PCP2 and Valparaíso -PCP3). Its antifungal effect against strain (ATCC 29213) of *Candida albicans* was evaluated. In addition, the botanical origin of pollen present in the sample was determined using the mesopalinological method.

For the preparation of the extract, 30g of Propolis were macerated with 100 mL of ethanol (70%). The content of polyphenols, anthocyanidins, total balms was quantified.

To quantify the antifungal activity, the method of quantification was used using foil disks containing 100  $\mu$ L of EEP for this purpose, the strain of *C. albicans* was sown in Petri plates containing nutrient agar media. As controls were used

The main plant species identified in the samples were: PCP1 (*Eucryphia cordifolia*; *Escanollia rubra*; *Aextoxicon punctatum*), PCP2 (*Quillaja saponaria*; *Lithraea quastica*; *Salix babylonica*) and PCP3 (*Maytenus boaria*; *Citrus lemon*, *Thymus vulgaris*)

The highest concentration of total polyphenols is obtained from the sample PCP1:  $41.365 \pm 3$  mg mL<sup>-1</sup>. For total anthocyanidins the sample PCP3 2.8 g L<sup>-1</sup>. Balms in PCP3 were 13.9 grams of balm. The average value of the inhibition halos showed significant differences when buying the inhibition halos versus the *C. albicans* strain ( $p < 0.05$ ) using the ANOVA test. In propolis, the inhibition halo was for PCP1:  $12.8 \pm 1.3$ , in sample PCP2:  $9.0 \pm 1.5$  and PCP3:  $10.1 \pm 2.0$ .

This study indicates that the extract of Propolis, has antifungal capacity and could be used as an adjuvant in the treatment of infections caused by *C. albicans*.

### PP-371

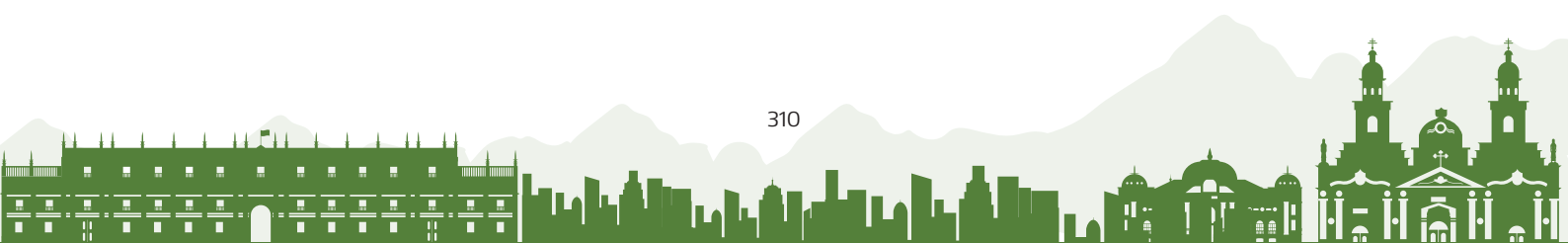
### Physicochemical characterization of the oily extract of carotenoids obtained from *Apis mellifera* pollen by supercritical fluids

Hector Suarez Mahecha<sup>1</sup>, Natalia Vilorio Pérez<sup>2</sup>, Lady Viviana Camargo Ovalle<sup>2</sup>

<sup>1</sup>Universidad Nacional de Colombia, Instituto de Ciencia y Tecnología de Alimentos ICTA, Colombia.

<sup>2</sup>Universidad Nacional de Colombia, Posgrado en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Agrarias, Colombia.

Bee pollen (*Apis mellifera*) is obtained from flower pollen collected by worker bees. It is called corbicular pollen because the corbicle or pollen basket is part of the tibia of the hind leg of bees and is there where it is transported to the hives. The objective of this work was to select the orange-yellow pollen by granulometry to obtain an oily extract of bee pollen using the supercritical fluid technique (SFE) using CO<sub>2</sub> as a solvent under supercritical conditions of pressure and temperature (P: 28MPa, Temperature: 60°C), Gas flow: 5L/min) and time of 6h (to obtain laboratory scale) and pressure conditions (P): 35MPa, Temperature (T): 45°C, flow (F): 50L/ min and time (t): 4hr for process in industrial equipment. Initially, pollen samples were collected from different municipalities in the department of Cundinamarca, Colombia (Guasca, Calera, Facatativá, Guatavita, Tenjo, Gachancipá, and Mosquera), to which the content of total carotenoids (CTC) and yield in the extraction process, these analyzes showed CCT values between 119 and 2140  $\mu$ g  $\beta$ -carotene/g with yield percentages in obtaining oily extracts between 3 – 5%. The samples with the best contents and yields were Guatavita, Tenjo, and Facatativá. For the CTC in the oily extract, results between 385 – 6942  $\mu$ g  $\beta$ -carotenoids/g of extract were obtained, with Guatavita pollen being the one with the highest content; For this reason, in this phase of the project, it is established that bee pollen from the Guatavita region could be used as a natural coloring for food. Furthermore, the pollen subjected to the SFE process presented positive affectation in the exine layers, evidenced in the analysis by scanning electron microscopy (SEM) because the final pollen samples showed high contents of total carotenoids, even after the process extraction.





### PP-372

### Egg enrichment with bioactive compounds obtained from bee pollen (*Apis mellifera*)

Hector Suarez Mahecha<sup>1</sup>, Natalia Johanna Viloria Pérez<sup>2</sup>, Lady Viviana Camargo Ovalle<sup>2</sup>

<sup>1</sup>Universidad Nacional de Colombia, Instituto de Ciencia y Tecnología de Alimentos ICTA, Colombia.

<sup>2</sup>Universidad Nacional de Colombia, Posgrado en Ciencia y Tecnología de Alimentos, Facultad de Ciencias Agrarias, Colombia

The coloration and contribution of bioactive compounds in the egg yolk are among the most important quality factors in the poultry industry. In addition, the effect of these components depends on the ingredients supplied in the diet of animals, due to the ability of the hens to metabolize the carotenoid molecules and deposit them in the yolk. This research aimed to evaluate the contribution of carotenoids in the egg through the inclusion in the diet of laying birds of oily extracts and orange/yellow granules of *Apis mellifera* bee pollen. The methodology included the elaboration of five treatments in the chicken diet, in which the source of carotenoids was modified: TT1: Negative control, TT2: Positive control commercial dye, TT3: Inclusion of oily extract of bee pollen, TT4: Inclusion of whole bee pollen selecting orange-yellow granules, TT5: Natural dye from *Tagetes* flower. For each treatment, samples of eggs were taken, and quality, nutritional, and sensory parameters were analyzed. For sensory and quality parameters (except yolk pigmentation), there were no significant differences ( $p \geq 0.05$ ) between the different treatments. Concerning nutritional parameters and those related to yolk color, the treatments presented significant differences ( $p \leq 0.05$ ), especially for TT3 oily pollen extract and red bee pollen granules TT4. The content of total carotenoids presented a significant difference ( $p \leq 0.05$ ) with values between 13.42-20.85  $\mu\text{g } \beta\text{-carotene/g}$  in the yolk, for the level of pigmentation ranges between 5-7 were obtained (based on the DSM 1-15 scale), for the color parameter based on the CieLAB scale, also presented significant difference ( $p \leq 0.05$ ) between the different treatments for the value of  $a^*$ . For the nutritional parameters, the values of the content of omega-3 fatty acids presented a significant difference ( $p \leq 0.05$ ) with values between 1.8-3.67, obtaining the highest range in the egg yolk from treatments 3 and 4. In addition, the oxidative stability of the same treatments also presented lower values than the other treatments. Statistical values show the relationship between these variables in the correlation and regression analysis carried out for the parameters of total carotenoid content, yolk color, fatty acid content, and oxidative stability.

### PP-373

### An approach to predicting honey production using Artificial Intelligence for beekeeping adaptation in response to climate change

Roberto Carlos Ahumada García, David Zabala-Blanco

Faculty of Engineering Science, Universidad Católica del Maule, Talca, Chile

Climate change has directly impacted the development of bees; drought, has negatively affected honey production by reducing the availability of water sources, nectar, and pollen, leading to a decline in these pollinating agents, which are essential for global food security. In Chile, an intense drought has been reported, harming beekeepers for over 10 years. Studies such as those by Gajardo-Rojas et al. (2022) establish a relationship between climatic variables like temperature and precipitation, and honey production.

Currently, beekeepers need predictive models focused on decision-making and adaptation to climate change. Therefore, this research proposes evaluating Artificial Intelligence models, such as extreme and multilayer neural networks, that can predict honey production based on climatic variables and various factors, including parasites, diseases, pesticides, nutrition, habitat, and beekeeping management.

In preliminary studies, the author has worked on the first approximation of flowering predictions for native Chilean trees, in research such as R. Ahumada & J. Reyes-Suarez (2016); and N. Grandón et al (2016). The methodology is based on training Artificial Intelligence models for learning from information about beekeepers' honey production and related climatic variables. The data used in these studies include: i) meteorological data such as temperature, rainfall, humidity, and radiation; and ii) historical records of honey production by beekeepers.

Preliminary results, have been obtained using information from the public database Kaggle (<https://www.kaggle.com/>), specifically: i) US Honey Production and ii) Climate Change: Earth Surface Temperature Data. The results indicate that, by using extreme and multilayer neural networks in conjunction with meteorological data, it is feasible to predict beekeepers' honey production. The prediction error of honey tends to decrease as the aforementioned algorithms continue learning. The authors aim to increase collaboration between universities and beekeepers in generating the necessary information for the efficient development of these predictive models, focusing primarily on Chilean and Latin American beekeeping.



### PP-374

### Melissopalynological analysis of honey from the Commune of La Unión, Los Ríos Region

Luisa Soto<sup>1</sup>, [Esteban Basoalto](#)<sup>1</sup>, Leticia Silvestre<sup>1</sup>, Karin Barrientos<sup>2</sup>

<sup>1</sup>Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Valdivia, Chile.

<sup>2</sup>Escuela de Graduados, Facultad de Ciencias Agrarias y Alimentarias, Universidad Austral de Chile, Valdivia, Chile.

Chile presents a great diversity of floral resources, making it important for developing Beekeeping. Nectar is among the most important resources for bees (*Apis mellifera* L.), the main source of carbohydrates from which honey is made. Honey is used for human consumption and, there are different classifications to describe its characteristics associated with its botanical origin. Within these, there is a classification according to their floral origin and honey can be classified as monofloral, bifloral and polyfloral. Today, honey typified by its botanical origin is in strong demand. Each type of honey, dominated by a type of nectar, allows us to differentiate by physicochemical, sensorial, and melissopalynological characteristics, the latter being the one addressed in this study. Through this characterization, the types of pollen determine the botanical origin of honey. For this reason, the melissopalynological attributes of honey from the commune of La Unión, Los Ríos Region, Chile were characterized. For this, pollen samples were obtained from honey from Mario Ruiz Vásquez apiary, sector of La Parrilla, Rio Bueno, harvested in the 2020 season. The average percentage of pollen for each botanical species was determined, according to the Chilean standard 2981 Of.2005, which classify as monofloral honey, honey with at least 45% of pollen grains of the same species. With this information, the relative abundance of the species was calculated. The results indicate the predominant presence of *Eucryphia cordifolia*, with a percentage greater than 80% of pollen grains, in all the samples studied.

### PP-375

### Using Advanced Technology for Hive Monitoring in order to improve Hive Management

[Rafael Cabrera](#)

President of Solutionbee LLC

Effective hive management is critical for reducing losses and optimizing honey production from healthy honeybee colonies. Traditional beekeeping methods involve physically inspecting hives to check for disease, swarming, queen health, and colony condition. However, emerging technologies are making it easier for beekeepers to monitor their hives and track the health of bees.

Advancements in communication technology, such as WiFi, Bluetooth, and NFC, as well as the development of AI for interpreting hive data sets, are creating new ways to monitor beehives. Several parameters, including weight, internal temperature, acoustics, and optics, can now be accurately measured by existing devices. Beekeepers interested in data science are using this information to make informed decisions about hive management.

Smart hive scales are becoming increasingly popular in the marketplace. These scales are placed under the hives and record weight and ambient temperature. By reading the hive weight automatically over regular intervals, beekeepers can obtain a running value and plot it over time. Just a few such scales in a beeyard can give a good picture of the overall activity in an entire apiary.

To reduce the communication cost of smart scales, one approach is to remove the cellular technology and instead use a WiFi hotspot to collect data from a larger number of hive scales. This approach can be implemented by using WiFi-enabled scales at a lower cost. The WiFi hotspot can also be used to connect other WiFi devices, such as cameras for security purposes and anti-theft prevention.

Internal temperature and humidity sensors can also upload data to the cloud via WiFi-enabled gateways. Real-time monitoring of the internal temperature of a hive can reliably warn beekeepers of queen loss and impending swarming.

Implementing affordable new technologies in beekeeping can significantly improve remote beehive monitoring with less intrusion than traditional beekeeping methods. This approach can be particularly beneficial for beekeepers in rural areas of the global south where beeyards may be distant and not easy to get to. By learning from early adopters and incorporating new tools, beekeepers can more effectively manage their hives and improve honey production.



PP-376

### Climate change and its impact on the production of honey in Slovenia

Aleš Bozovičar

Slovenian Beekeepers Association, Lukovica, Slovenia

As evident, the temperature has been rising in Slovenia in the last decade. We, beekeepers, have been noticing premature vegetation and blossoming of plants and longer periods of no grazing for the bees during the summertime, as a result of milder winters with minimal to no/zero snowfall. Plants that have been frostbitten sustain immediate damage, which can be seen a few days after the frost with the death of the flower shoots followed by poorer flowering and no grazing for the bees. The consequences of climate change are also reflected in the altered precipitation patterns in the country. Slovenia has been experiencing longer periods of rainfall, especially during the best months for honey production - May and June. These prevent the bees from leaving their hives during the rain to collect pollen from the nature. The shortage of pollen results in weaker development of the bee colonies and ultimately lower production of honey during the subsequent grazing period. Frosts in the month of April can also cause great damage to honeydew agents, especially *Physokermes piceae* Schrank on smreka. Low temperatures do not harm them when they are dormant, but with mild winters and higher average temperatures they start to develop prematurely resulting in hypothermia and subsequent damage. Forests play an important role in Slovenia, as they cover more than 58% of its surface. Therefore, they also play a vital role in beekeeping. Honey producing plants in Slovenia, smreka (*Picea abies*) and jelka (*Abies alba*), can provide excellent grazing for the bees during the favourable year/s. Climate change and global warming have been having adverse effects in our forests. In addition to more frequent natural disasters (ice rains, windstorms, forest fires), we have been recording smreka with an extensive pest infestation, reducing the proportion of conifers. The same scenario is also to be predicted/expected in the future where the climatic conditions will be more favourable mainly for the growth of leafy species of plants, which unfortunately do not offer anything worth mentioning to the bees.

PP-377

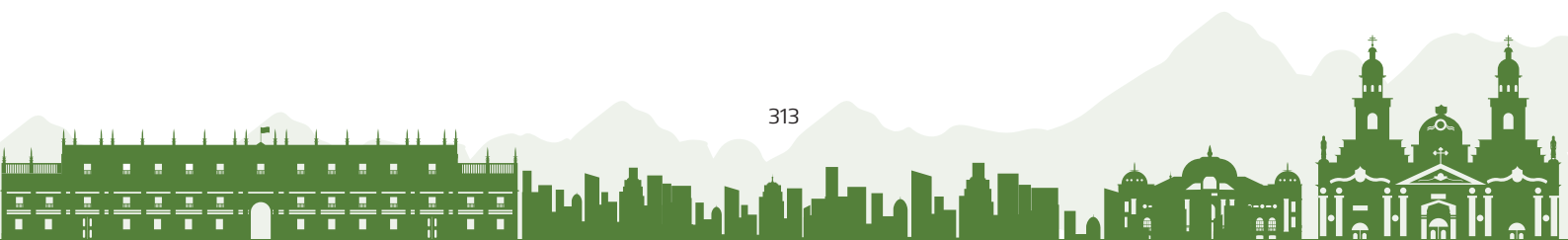
### Determinación de la actividad antibacteriana y antifúngica in vitro del propóleo proveniente de tres regiones ecuatorianas

Luis Rodrigo Galarza

Universidad de Cuenca, Facultad de Ciencias Agropecuarias, Carrera de Medicina Veterinaria

Determinación de la actividad antibacteriana y antifúngica in vitro del propóleo proveniente de tres regiones ecuatorianas.

The indiscriminate use of antimicrobials in animals caused a growing antimicrobial resistance, the alternative use of propolis is effective against resistant microorganisms because it has flavonoids and phenolic components. The objective was to determine the antibacterial and antifungal activity in vitro of propolis coming from three Ecuadorian regions (Costa, Sierra and Oriente). Initially, we prepared the ethanol extract of propolis (EEP) to 20% of each region. The microorganisms *Staphylococcus aureus* and *Malassezia* spp., were identified, isolated and cropped in vitro of canine otitis patients. This study has two experiments each one had 5 treatments and 7 repetitions: EEP-Costa, EEP-Sierra, EEP-Oriente, antimicrobial (gentamicin or fluconazole) and control (alcohol). The effect of each treatment was valued in halos of inhibition of antibiogram (Experiment 1) and antifungigram (Experiment 2). In the experiment 1, gentamicin produced major ( $P < 0,01$ ) halo of inhibition ( $25,2 \pm 0,46$  mm). But, the treatments of EEP produced major ( $P < 0,01$ ) halos of inhibition against the control, getting the best results the EEP-Oriente with the major ( $P < 0,01$ ) halo of inhibition ( $23,2 \pm 0,43$  mm). In the experiment 2, all the EEP produced major ( $P < 0,01$ ) halos of inhibition against the fluconazole and control. Nevertheless, the best treatment was the EEP-Sierra with major ( $P < 0,01$ ) halo of inhibition ( $26,1 \pm 0,45$  mm). In conclusion, the Ecuadorian EEP to 20% performs effectively its antibiotic and antifungal action. In addition, the EEP-Sierra and EEP-Oriente produced best antimicrobial effects than the EEP-Costa. Therefore, the recommendation is to validate this antimicrobial effects in vivo in patients with local infections.



## Beekeeping Technology and Quality

### PP-378

#### Protocol for the collection of authentic honey samples for Fluorescent Spectroscopy Testing

Lynne Ingram<sup>1</sup>, Alex Rozhin<sup>2</sup>, Raghavan Chinnambedu Murugesan<sup>2</sup>, Steven Daniels<sup>2</sup>, Valentina Barker<sup>2</sup>

<sup>1</sup>Honey Authenticity Network UK

<sup>2</sup>Aston Institute of Photonics Technology (AIPT) Electrical and Electronic Engineering College of Engineering and Physical Sciences, Birmingham, UK.

The Honey Authenticity Network UK (HAN UK) is an organisation of beekeepers who are working together to raise awareness of the issues of honey adulteration and fraud in the UK and beyond.

HAN UK is now working in collaboration with scientists from Aston University, on the development of a new test, using fluorescent spectroscopy, to detect the adulteration of honey. Whilst research is in its early stages, we are ultimately working towards this test being used from a portable hand-held device using a mobile app.

A database of authenticated honey samples is being developed, and we are keen to see this database being accessible to all who need to use it. Problems have arisen in the past due to limitations arising from issues of ownership.

We will present a protocol to authenticate samples for this database that works for both scientists and beekeepers, and discuss issues of access and ownership.

### PP-379

#### Could plastic be a truly sustainable alternative to wooden beehives in world of climate change?

Paula Carnell

Paula Carnell Limited

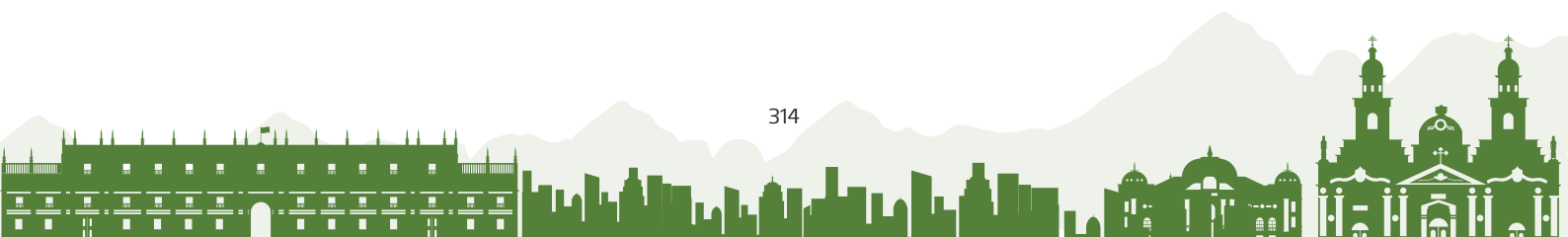
Determinación de la actividad antibacteriana y antifúngica in vitro del propóleo proveniente de tres regiones ecuatorianas.

With natural beekeeping we tend to favour using wood to build beehives. As much of the world is becoming deforested, and with trees providing much needed forage for bees, should we be looking at other materials for building bee hives if there is not an abundance of felled trunks?

As a natural beekeeper I was always favouring wood for building hives, or straw for weaving skeps. In a commercial situation however hives need to be easily built from a readily available resource. When flow hives first came onto the market, there was much discussion about the viability of plastic within a hive and whether or not the bees would adapt to it. Health concerns were also raised about the use of plastic and its replacement of wax foundation due to the valuable properties of wax within the colony.

Many commercial honey producers have been using plastic foundation due to its ease of stability during the honey extracting process. I was not an advocate of using plastic beehives due to the negative connotations of the production and disposal of plastic materials.

However, when visiting the Cocos Keeling islands and working with Jack Clunies Ross and his bees, I could see the benefit of plastic hives, namely the Apimaye hive in a tropical climate. The remote location and humid conditions of Cocos Keeling make the shipping in of wooden hives unfavourable in both cost and longevity as the wood rots and becomes mouldy very quickly, not beneficial to bee health. Using Flow hives was favourable due to the sheer volume of honey the bees on Cocos produce in the year round abundant and pristine environment. The fully plastic hives however proved economically sound due to their resistance to humidity and enabling the bees space and insulation to maintain their hives for many years. The nature of plastic is that it literally lasts forever, and so the question of disposal doesn't come into the equation. With Cocos Keeling's fame being of tonnes of washed up single use plastic along its reef, could plastic beehives be an ironic twist in their beekeeping industry?



### PP-380

### MEDIBEES: Monitoring the Mediterranean honey bee subspecies and their resilience to climate change for the improvement of sustainable agro-ecosystems

Raquel Martín Hernández<sup>1</sup>, Antonio Nanetti<sup>2</sup>, Maria Alice Pinto<sup>3</sup>, Adjlane Nouredine<sup>4</sup>, Nizar Haddad<sup>5</sup>, Chadi Hosri<sup>6</sup>, Mustafa Necati MUZ<sup>7</sup>, Marion Zammit Mangion<sup>8</sup>, Ahmad Yousef<sup>9</sup>, Soledad Sagastume<sup>1</sup>, Giovanni Cilia<sup>2</sup>, Medibeec Consortium<sup>10</sup>

<sup>1</sup>CIAPA-IRIAF Centro de Investigación Apícola de Marchamalo, Marchamalo, Spain

<sup>2</sup>CREA-AA Centro di Ricerca Agricoltura e Ambiente, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Bologna, Italy

<sup>3</sup>CIMO Mountain Research Centre, Polytechnic Institute of Bragança, Campus de Sta. Apolónia, Bragança, Portugal

<sup>4</sup>UMBB-DZ M'Hamed Bougara University of Boumerdès, Faculty of Science, Boumerdès, Algeria

<sup>5</sup>NARC National Agricultural Research Center, Amman, Jordan

<sup>6</sup>UL Lebanese University, Faculty of Agriculture, Beirut, Lebanon

<sup>7</sup>UNK University of Namik Kemal, Faculty of Veterinary Medicine, Tekirdağ, Turkey

<sup>8</sup>UM Department of Physiology and Biochemistry, University of Malta, Msida, Malta

<sup>9</sup>JBU Jordanian Beekeepers Union, Jordan

<sup>10</sup>Various institutions

Beekeeping provides livelihood for hundreds of thousands of beekeepers in the Mediterranean area. This activity relies on a number of different indigenous *Apis mellifera* subspecies, adapted to the very diverse and harsh conditions of the region. Climate change is expected to increase the stress factors affecting bees, especially in this region, reducing both pollination efficiency and production potential. Unfortunately, our ability to address this problem is limited by the incomplete knowledge of the natural adaptation mechanisms developed by the different subspecies. In order to increase the knowledge base for future selection programs to improve bee populations for environmental changes, the MEDIBEES project is being developed. It includes 9 partners from 8 Mediterranean countries on all three shores of the Mediterranean, covering 10 local *A. mellifera* subspecies, which represents a remarkable though understudied proportion of the species genetic diversity. The project aims to: a) unravel the differential genetic background of Mediterranean subspecies, b) understand their adaptation to local conditions, and c) characterize their resistance to climate change. To achieve the objectives, colonies belonging to the local subspecies will be studied phenotypically to determine their behavior under environmental conditions, covering survival, sensitivity to pests/pathogens, behavior, physiology and reproduction which will be completed by gene expression and transcriptomic assays. In addition, complete genomes of field and laboratory samples will be sequenced to find genes putatively involved in adaptation and to develop new genetic tools to characterize honey bee populations according to their resistance to environmental stress factors. This effort will encourage the use of local subspecies, to make them more attractive and avoid importing foreign breeds, and will lay the foundation for future selection programs. Besides, the valorization of honey by both promoting its use and developing quality labels, and the evaluation of beekeeping by-products as modifiers of soil fertility and biota are also approached to help the beekeepers improve the sustainability of their farms in an economical and environmental sound manner.

This project is part of the PRIMA programme supported by the European Union.

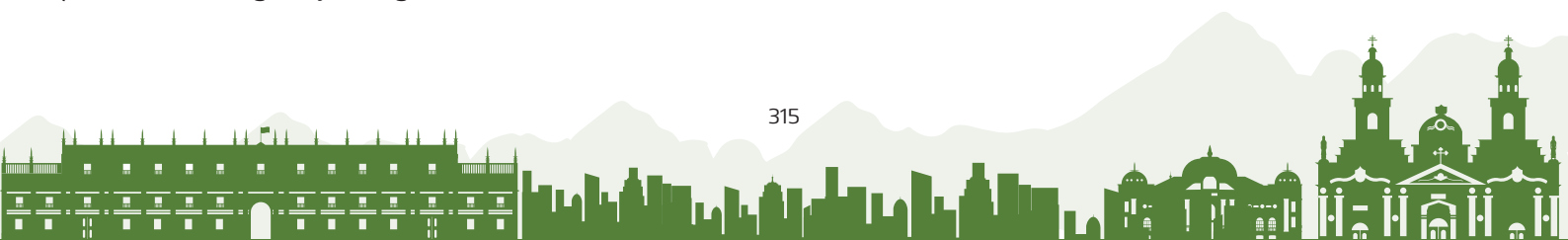
### PP-381

### Accuracy analysis of two evaluation methods for the area of capped brood in honey bees hives

Nicole Rosenstock, Soledad Lorenzo, Kaharen Del Castillo, Sofía De León, Enrique Nogueira

Facultad de Veterinaria - Universidad de la República

Evaluation of colony development is usually conducted at the beginning or during an experiment when the hives are standardized and/or at the end as response variables. Adult bees in a colony vary in number according to the time of day, weather conditions or resource availability. Therefore, evaluating the brood is more reliable and can be accomplished through a) objective methods (OM), such as analysis of brood area with ImageJ, and b) subjective methods (SM) where one or more operators estimate the brood area while observing the hive. OM are precise but require a lot of time for image analysis. SM are quicker but could be imprecise and/or inexact. Recently, a semi-automatic method called CombCount (CC) was developed, which allows quick image analysis. Our goal was to evaluate the precision of the CC and SM in the measurement of the area of capped brood. Four hives with capped brood were used: they were photographed and estimated by 2 trained operators. Images were analyzed with ImageJ and these results were compared against those obtained by CC and SM, and were analyzed statistically. In the comparison between CC and ImageJ, the regression line ( $y=0.8585x + 0.2023$ ,  $R^2=0.962$ ) is closer to the ideal of perfect agreement ( $y=x$ ), than the MS vs ImageJ regression line ( $y=1.2033x + 0.8837$ ,  $R^2=0.967$ ). Lin's coefficient, an index that evaluates the concordance between measurement methods, yielded a "substantial" value (McBride, 2005) of 0.9627 for the first analysis. For the second analysis, a "moderate" Lin coefficient (McBride, 2005) of 0.9384 was obtained. The parameters that measure the precision of the method (Cb- bias correction factor, Location shift, Scale shift) were better for CC, and the difference in precision ( $r$ ) between both groups was minimal. Comparing the two Lin's coefficients, it can be observed that CC has a slightly higher level of concordance with the Gold Standard ImageJ than SM. Both methods, CC and SM, can be used alone or in combination during an experiment: CC eliminates the main disadvantage of the OM, which is the time required for an analysis, while SM with trained operators are more precise than originally thought.





### PP-382

## Characterization Of Apolar Extracts Of Colombian Propolis (Aecps) And Their Correlation With The Antimicrobial Activity Of Gram Positive And Negative Bacteria

David Guillermo Piedrahita Marquez<sup>1</sup>, Lady Viviana Camargo Ovalle<sup>2</sup>, Marcelo Maraschin<sup>3</sup>, Caroline Schmitz<sup>4</sup>, Consuelo Díaz-Moreno<sup>5</sup>, Hector Suarez Mahecha<sup>6</sup>

<sup>1</sup>David Guillermo Piedrahita Marquez

<sup>2</sup>Lady Viviana Camargo Ovalle

<sup>3</sup>Caroline Schmitz

<sup>4</sup>Marcelo Maraschin

<sup>5</sup>Consuelo Díaz-Moreno

<sup>6</sup>Héctor Suárez-Mahecha

Honeybees produce numerous components beyond the honey, which have a notorious bioactivity against gram positive and gram negative bacteria found in food products. One of them is the propolis, which are produced by the recollection and transformation of the plant exudates on the beehive, and their composition and action against pathogenic microorganisms varies according to the hive structure and the plant chemical composition. In the literature there is a lack of information of the Colombian propolis action against pathogens and there is no information about the chemical profile of the compounds in these samples, therefore, to identify the chemical profile of propolis and to correlate the metabolites with the antimicrobial action is necessary to perform a metabolomic analysis which integrates spectroscopic techniques and bioassays. To obtain the profile and concentration of low molecular weight metabolites, amino acids, fatty acids and polyphenols it was used techniques such as GC-FID and NMR, then, the extracts were analyzed by the minimum inhibitory concentration (MIC) tests to elucidate the bactericidal power of the extracts. It was concluded that the ecosystem and the botanic species are the main characteristics that allow the discrimination of the propolis, also it was seen that the bioactivity against pathogens was due to a synergistic effect between various metabolites which have variations in their chemical structure, mainly on the molecular weight and the functional groups. Also it was seen that there is a bigger influence of the ecosystem on the propolis composition in comparison to the bee species that collects the exudates, the red propolis had a higher bioactivity than the other types of propolis because they are going to have more exudates from different plants which are going to include crops like guava and coffee and species from the Eucalyptus, Clusia and Dalbergia genus

### PP-383

## The simple way to obtain a large amount of worker bees (foragers)

Guram Rtskhiiladze<sup>1</sup>, Irakli Rtskhiiladze<sup>3</sup>, Shalva Rtskhiiladze<sup>2</sup>

<sup>1</sup>Guram Rtskhiiladze

<sup>2</sup>Irakli Rtskhiiladze

<sup>3</sup>Shalva Rtskhiiladze

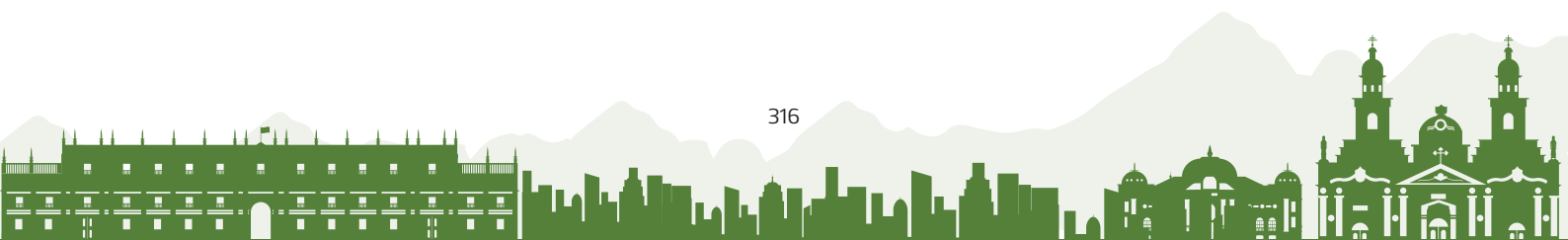
Over the years, there have been attempts by beekeepers in several countries to obtain a large amount of forager bees by having concentrated them in one hive.

In normal condition bees can distinguish another colony member and don't let them in hive. In early spring when fruit blossoms and during a honey flow, ability to distinguish foreign bees is diminished; When the hives are lined up in a straight row and kept close enough together, some returning bees are disoriented and drift towards the neighbor hives, more frequently to the end of the rows. Those hives at the end of the rows will become stronger than those in the center and this colony produces a surplus of honey. This was established by American beekeepers D. Sammataro and A. Avitabile.

Another important point is that returning forager bees when returning from fields loaded with nectar or pollen could with ease and without obstacles such as guarding bees enter neighbor hives.

Based on these principles it is possible to concentrate huge amount of forager bees in one hive just moving hives in special order.

Proposed method is a simple, essentially different from others, due to: we do not shake or brush bees from frames or add frames full of eggs and sealed brood; we neither have a combination of multiple queens in one hive, or device with queen excluder, which placed on top of the brood nest to prevent the queen from entering the honey supers above, or shake swarms in supers above queen excluder. We do not even need to examine stock before manipulation. Realization of our system is easy: proceeds successfully and at the same time this whole process takes only minutes, with no harm to the bees as well as beekeepers. The best time for starting and accomplishing the suggested scheme of manipulation is early spring, period of honey flow which coincides in republic of Georgia with April, May and two first weeks of June.



### PP-384 Llaxacondor confinement cage

Javier Llaxacondor

Abejas del Perú

The Llaxacondor Confinement Cage is useful for queen rearing in cold environments. This experience has been successfully tested in Peruvian hives that were installed over 2,800 meters above mean sea level with European and Africanized bees.

This confinement cage holds 4 frames and a queen rearing frames with up to 90 plastic or wax cell cups. It is placed in the super of a vigorous 2-storey queenright colony and it performs as the cell starter. The 90 queen cells are easily accepted during the first 24 hours, which is the time that the confinement of 1.5 kg of young bees lasts. The density and number of bees is key to assure good feeding (quantitatively and qualitatively), and optimal humidity and temperature for the larvae. Grafting can be done one more time after the accepted cell cups have been placed in the finisher colonies. Afterwards, the confined colony can be used to make mating nucs or to reinforce a weak colony in the apiary.

The main innovation of the Llaxacondor confinement cage is that it enables to have a “starter colony within the same colony”, taking advantage of the colony as a “natural incubator” and counteracting the disadvantageous low temperatures in cold environments. In addition to this, it optimizes time compared to making and dissolving a regular “starter colony”.

Last but not least, another advantage of this confinement cage is that it can be easily replicated by any beekeeper and at a very low cost, which is specially beneficial for low-income countries. The materials to build one of these cages are very common and easy to find: small pieces of wood, a small tin sheet and a small piece of mesh. The full presentation will include more details of the construction of the confinement cage, how to use it and the results obtained in the Peruvian context.

### PP-385 Thermal degradation study of red propolis microspheres with sodium alginate by extrusion method

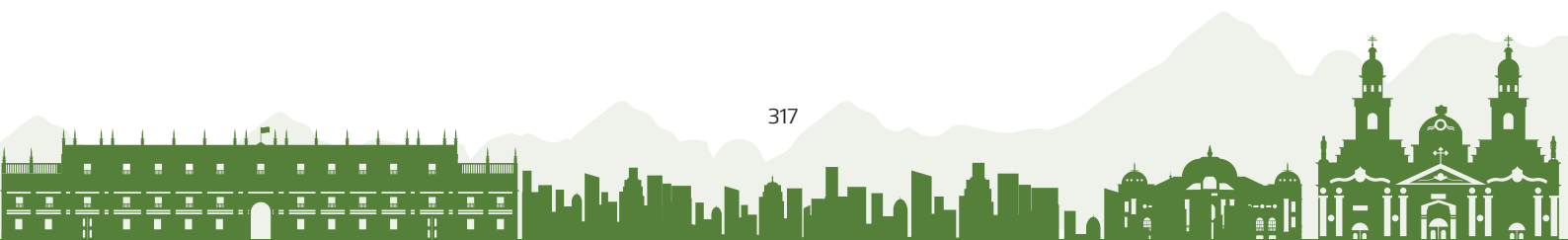
Clara Andrezza Crisóstomo Bezerra Costa<sup>1</sup>, Jeniffer Mclaine Duarte De Freitas<sup>1</sup>, Alan John Duarte De Freitas<sup>1</sup>, Davdson Luiz De Souza<sup>2</sup>, Johnnatan Duarte De Freitas<sup>1</sup>, Edeildo Ferreira Da Silva Júnior<sup>3</sup>, Ticiano Gomes Do Nascimento<sup>2</sup>

<sup>1</sup>Department of Chemistry, Federal Institute of Alagoas, Maceió, Brazil

<sup>2</sup>Institute of Pharmaceutical Sciences, Federal University of Alagoas, Maceió - Brazil

<sup>3</sup>Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió - Brazil

Propolis is produced by bees, from the collection of resins and flower buds. These insects carry out a partial digestion of this collected material, and add beeswax. Red propolis had its origin evidenced in the Northeast region, precisely on the coast of Alagoas. Flavonoids and phenolic acids are the bioactive compounds responsible for the therapeutic properties and these biological activities have antiviral, antifungal, anticancer, immunomodulatory, anti-inflammatory, healing, antioxidant and antimicrobial action. The interest of the food, pharmaceutical and cosmetic industries in the application of particles in gels has increased in recent years. This is because the microencapsulation technique allows preventing the loss of chemical substances via natural processes, such as volatilization and oxidation, preserving all the physicochemical characteristics of the processed material. The objective was to develop red propolis microspheres by the extrusion method with sodium alginate and calcium chloride. The method used was based on ionic gelation of sodium alginate with calcium chloride. Thus, the obtained material was characterized by Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC) to study thermal degradation. The results were based on the best formulation of the microspheres. From this, in TG, there was an initial loss of 15.014% (0.895 mg) of mass until reaching a temperature of 209.38 °C and, after mass loss of 46.083% (2.74 mg), referring to the temperature variation of 209 to 601.20°C. Finally, upon reaching 898.94 °C, a third mass loss of 10.334% (0.616 mg) is observed. In the DSC, an initial exothermic event was verified at ~90 °C. At 197 °C, an endothermic event is observed, compatible with the glass transition of the material and, between 250 and 300 °C, a subtle endothermic event is observed referring to the decomposition of the constituents of the microspheres, as well as, it is possible to verify the presence of a very high peak. accentuated for endothermic events between 500 and 570 °C. Therefore, the results demonstrated good stability of the material, confirming that the microspheres are attractive for the use of nanocarriers of active substances.



### PP-386

#### Characterization by solid phase microextraction of volatile compounds from red propolis produced in Marechal Deodoro, Alagoas, Brazil

Clara Andrezza Crisóstomo Bezerra Costa<sup>1</sup>, Jeniffer Mclaine Duarte De Freitas<sup>1</sup>, Orlando Francisco Da Silva Moura<sup>1</sup>, Alan Jonh Duarte De Freitas<sup>1</sup>, Jório Bezerra Cabral Júnior<sup>4</sup>, Ticiano Gomes Do Nascimento<sup>2</sup>, Johnnatan Duarte De Freitas<sup>1</sup>, Celso Silva Caldas<sup>1</sup>, Edeildo Ferreira Da Silva Júnior<sup>3</sup>

<sup>1</sup>Department of Chemistry, Federal Institute of Alagoas, Maceió, Brazil

<sup>2</sup>Institute of Pharmaceutical Sciences, Federal University of Alagoas, Maceió - Brazil

<sup>3</sup>Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió - Brazil

<sup>4</sup>Department of Geography and Environment, Federal University of Alagoas, Maceió - Brazil

Propolis is a resinous, gummy and balsamic substance produced by bees by collecting resins, flowers and plant buds. Bees mix the collected resin with their salivary secretions, partially digesting it and adding beeswax, thus forming propolis. The main bioactive compounds present in propolis are phenolic compounds, terpenoids and steroids, depending on their constituents, propolis has several pharmacological actions, highlighting the antioxidant and antimicrobial properties. The aim was to study the solid phase microextraction (SPME) of volatile compounds from red propolis produced in Marechal Deodoro-AL. The research took place in the Chemistry laboratory of the Federal Institute of Alagoas IFAL Campus Maceió. For the extraction of volatiles, 5g of crude solid propolis were placed in a round bottom flask closed with a rubber septum and the SPME fiber exposed for 1h. The system temperature was maintained at 40°C using a water bath. After extraction, SPME fiber was inserted into the Gas Chromatograph injector coupled to Mass Spectrometry (GCMS) and the extracted compounds were analyzed in the programmed methodology. The three main compounds identified were: Trans-alpha-bergamotene (26.54%), Anethole (16.19%) and Biciclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene -, [1R- (1R \*, 4Z, 9S \*)](14.23%). The chromatographic results of microextraction by solid phase allowed identifying the bioactive compounds present in red propolis from Marechal Deodoro-AL, with the purpose of knowing these chemical substances for the development of new drugs that have biological actions against pathologies or microorganisms resistant to current antibiotics.

### PP-387

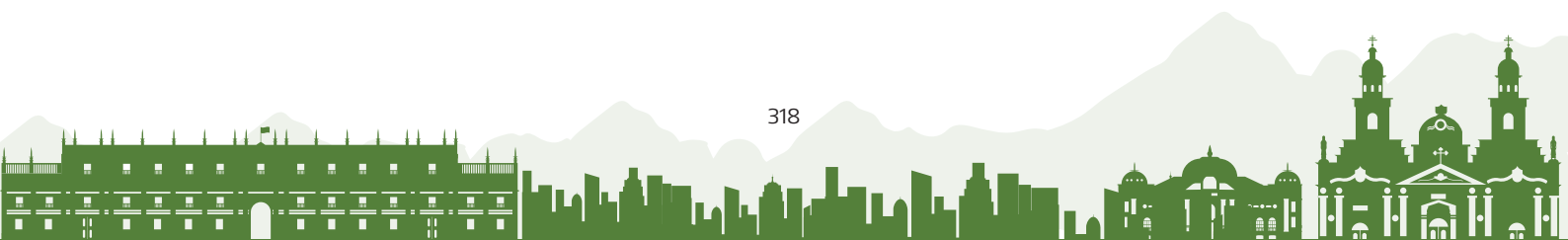
#### Physicochemical characterization of honey sold in Maceió, Alagoas, Brazil

Clara Andrezza Crisóstomo Bezerra Costa<sup>1</sup>, Jeniffer Mclaine Duarte De Freitas<sup>1</sup>, Alan John Duarte De Freitas<sup>1</sup>, Mikael De Lima Freitas<sup>1</sup>, Demétrius Pereira Morilla<sup>1</sup>, Ticiano Gomes Do Nascimento<sup>2</sup>, Johnnatan Duarte De Freitas<sup>1</sup>

<sup>1</sup>Department of Chemistry, Federal Institute of Alagoas, Maceió, Brazil

<sup>2</sup>Institute of Pharmaceutical Sciences, Federal University of Alagoas, Maceió - Brazil

Honey is considered one of nature's purest foods, appreciated for its characteristic flavor and considerable nutritional value, its price is relatively high, which often encourages adulteration. Systematically, beekeepers and consumers have shown great concern with the quality of honeys. In this sense, physical-chemical analyzes were carried out on samples of honey from 20 apiaries in the city of Maceió-AL. The analyzes aimed to detect possible fraud and/or adulteration of honey, such as the addition of corn syrup, beetroot and inverted syrup, thus causing changes in its nutritional value. To carry out the, the guidelines and methodology recommended by the Ministry of Agriculture and Supply, through the Normative Instruction of 2000, were followed, with analyzes of hydrogenion potential, hydroxymethylfurfural, acidity, formaldehyde index, humidity, ash, soluble solids, reaction Lund's, Fiehe's reaction, amylase index, reducing sugars, total reducing sugars and color. All analyzed samples presented quality parameters within the standards established by current legislation and, therefore, suitable for consumption by the population, ruling out possible adulteration of products sold in Maceió-AL.



### PP-388 Monitoring Bee Activity Using RFID Technology

Vojtěch Purnoch<sup>1</sup>, Dalibor Titera<sup>1</sup>, Martin Kamler<sup>1</sup>, Jan Kazda<sup>2</sup>

<sup>1</sup>Bee Research Institute, Dol, Czech Republic

<sup>2</sup>Czech University of Life Sciences Prague, Prague, Czech Republic

For a long time, we have relied only on direct observation for insect monitoring, which was not only time-consuming but also potentially inaccurate. In the last decade, however, we have seen the incorporation of technologies such as radio frequency identification (RFID). RFID systems in honey bees are primarily used to monitor the effects of pesticides and pathogens on bee flight and longevity. To a lesser extent, the technology has been used to better understand the plasticity of foraging behaviour. The system is based on the use of passive chips without their own power supply and a set of monitoring gates with a sensor. The chips are so lightweight due to the absence of a battery that the bees can easily carry them. For monitoring purposes, they appear to be an ideal tool. The pilot experiments of the project prevented a good interpretation of the results. However, the project has achieved partial results after improving the methodology and adapting the technology. At present, the experiments are being carried out at 4 sites, each with 4 colonies. The RFID system shows the influence of biotic and abiotic environmental conditions on the flight activity of bees. So far, the effect of the application of plant protection products on the flight activity of chipped bees has not been demonstrated at any of the sites. However, the flight activity of a colony is linked to its internal conditions and changes in these conditions may indicate changes in the surrounding environment. Thus, monitoring of colony activity could be a tool for monitoring environmental conditions alone or in combination with other methods.

The study is made possible and funded by the TAČR project SS03010178.

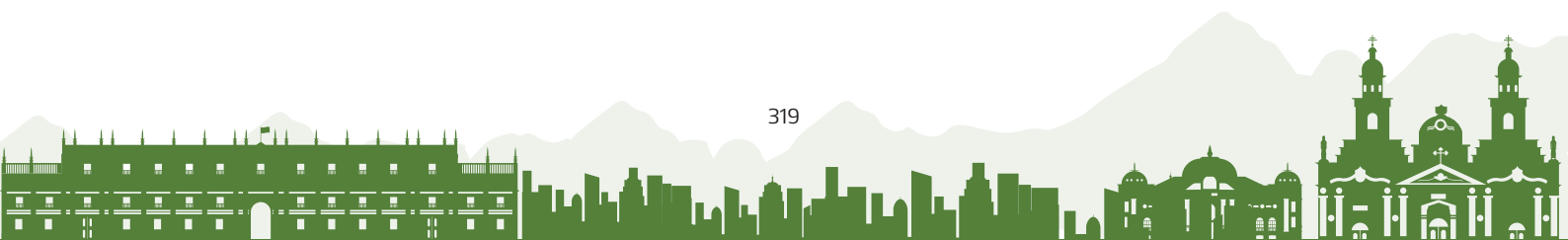
### PP-389 Biological value of Ukrainian sunflower honey

Leonora Adamchuk<sup>1</sup>, Artem Antoniv<sup>2</sup>

<sup>1</sup>National University of Life and Environmental Sciences of Ukraine, National Scientific Center «P.I. Prokopovich Institute of Beekeeping», Public Organization «Foundation of Women Beekeepers», Kyiv, Ukraine

<sup>2</sup>National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

The biological value of sunflower honey depends on its botanical purity, geographical origin, and physicochemical properties, such as enzyme content, free acidity, electrical conductivity, and antioxidant activity. The enzyme activity of honey is characterized by diastase and proline. Sunflower honey collected from different regions of Ukraine had varying levels of diastase and proline: Kyiv (24.2±2.54 Gothe units and 542.0±0.32 mg/kg), Chernihiv (17.6±1.04 and 364.0±0.21), Cherkasy (14.6±1.01 and 302.0±0.56), Kropyvnytskyi (39.9±1.05 and 435.0±0.73), Kherson (20.4±3.82 and 573.0±0.34), Mykolaiv (15.9±3.17 and 665.0±0.35), Dnipro (16.4±2.59 and 347.0±0.72), Zaporizhzhia (18.5±2.02 and 647.0±0.35), and Odesa (23.4±1.02 and 725.0±0.24). Sunflower honey collected in Kyiv region has an acidity of 10.8±0.34 mEq/kg and an electrical conductivity of 0.2±0.01 mS/cm; in Chernihiv - 15.6±0.15 and 0.6±0.02; Cherkasy - 19.5±0.51 and 0.5±0.01; Kropyvnytskyi - 15.3±0.05 and 0.3±0.01; Kherson - 13.9±0.33 and 0.3±0.01; Mykolaiv - 14.8±0.32 and 0.5±0.02; Dnipro - 13.6±0.13 and 0.3±0.02; Zaporizhzhia - 15.7±0.34 and 0.8±0.05; Odesa - 12.5±0.11 and 0.2±0.02. Adding sunflower honey to tea made from *Sambucus nigra* L. berries increases its antioxidant activity. The antioxidant activity of the tea without honey was 27.27 units, which increased to 52.07±0.556 with the addition of sunflower honey from the Eastern Steppe of Ukraine, 53.80±0.322 from the Forest-Steppe, 57.55±0.273 from Polissia, and 55.67±0.268 from the Eastern Steppe. Sunflower honey was found to be most effective against *E. coli*, inhibiting its growth at a concentration of 0.188 g/ml. Sunflower honey with over 90% monoflorality inhibited the growth of *Sal. enterica* at a concentration of 0.094 g/ml, while honey with lower monoflorality inhibited its growth at a concentration of 0.188 g/ml or 0.375 g/ml. Sunflower honey inhibits the growth of *S. aureus* at a concentration of 0.094 g/ml. With a lower degree of monoflority at a concentration of 0.188 g/ml or 0.375 g/ml. Sunflower honey inhibits the growth of *S. aureus* at a concentration of 0.094 g/ml. Compared to other studied varieties of honey, sunflower honey was most effective against all studied strains of bacteria ( $p=0.01$ ). Sunflower honey has a bactericidal effect at a concentration of 0.188 g/ml for *E. coli* and *L. monocytogenes*, 0.75 g/ml for *Sal. enterica*, *S. aureus*.



### PP-390

#### Physical, chemical and sensory quality of propolis collected in Cuenca del Salado region, Argentina

María Soledad García Paoloni<sup>1</sup>, Irene Cibanal<sup>2</sup>, Leticia Fernández<sup>2</sup>, Sivana Rodriguez<sup>3</sup>, Ana Paula Murray<sup>3</sup>, Liliana Gallez<sup>2</sup>

<sup>1</sup>-Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Hilario Ascasubi, Argentina

<sup>2</sup>LabEA UNS-CIC, Departamento de Agronomía, Universidad Nacional del Sur, Bahía Blanca, Argentina

<sup>3</sup>INQUISUR-CONICET, Departamento de Química, Universidad Nacional del Sur, Bahía Blanca, Argentina

The "Cluster Apícola de la Cuenca del Salado" (CACS) is a non-profit organization that brings together approximately one hundred beekeepers from the east of Buenos Aires province, Argentina. In this area, the existence of trees such as eucalyptus, acacias, willows and casuarinas, among others, offer a suitable source of resins for the propolis production. To carry out a diversification and added-value project, the CACS organized technical training for the beekeepers, and purchased propolis traps, which were set in hives from ten apiaries distributed nearby the cities of Rauch (3), Chascomús (2) and Mar del Plata (5) between November 2021 and March 2022. Sensory analyses were performed by a panel of seven semi-trained evaluators who described the presentation, appearance, brightness, colour, consistency, intensity and description of the smell, taste and trigeminal sensations, as well as aromatic intensity. Also were considered the presence of mechanical impurities. The analyzed physico-chemical parameters were waxes, total polyphenols/flavonoids content, oxidation index, UV spectrogram (IRAM-INTA norms 15935-1/2) and colour (CR-400 Chroma meter Konica Minolta). The general appearance of the samples was homogeneous, with a scarce presence of impurities and a predominant bright reddish-brown colour, corroborated by CIE L\*a\*b\* system. All the samples presented a hard to semi-hard consistency at 20 + 4°C, a mild resinous odour with slight differences in intensity and insipid flavour. The analysis of the raw samples showed differences regarding the wax content from 9.82 to 40.74%. All alcoholic-extracts exhibited a characteristic absorption profile in the UV-visible spectrum with a wide broad band between 240 and 340 nm and maximum values between 290/300 nm, which is characteristic of phenolic compounds. The extracts also differed in the content of total polyphenols (50.09-86.98 g eq gallic acid/100 g propolis), total flavonoids (1.97-5.22 g eq quercetin/100 g propolis) and oxidation index (1.0-7.0 seconds). All samples showed values in accordance with the quality standards required by Argentina regulations, except for only one from the coastal area that exceed 0.74% of wax content. Thus, it can be concluded that all samples exhibited excellent sensory, physical and chemical characteristics, which makes the studied area promising for propolis production.

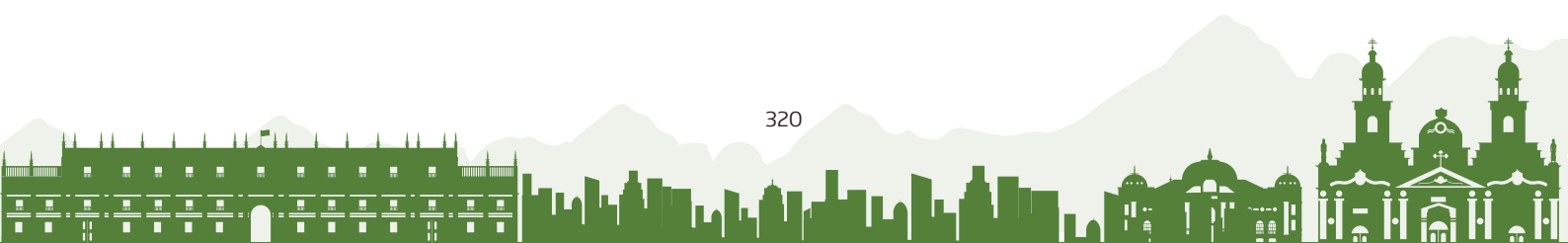
### PP-391

#### Valorisation of Beehive by-Products obtained from an apiary at Central Spain

J. Antonio López-Pérez, Amelia V. González-Porto, Carlos García Villarrubia, Mariano Higes, Soledad Sagastume De Andrés, Raquel Martín Hernández

Centro de Investigación Apícola y Agroambiental (CIAPA) de Marchamalo - Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal (IRAF)

A pre-liminary study of beehive by-products has been carried out. The aim of this study is to find out its potential as a local source of organic matter to be used as a soil improver and an organic source for soil biodisinfestation able to reduce soil phyto-pathogens. The content of these by-products is mainly wax, pollen, honey, propolis, feces, remains of dead bees or of possible intruders that appear in the bottom of the beehive. This composed by-products were generated as result of the beekeeping activity in an apiary located in Central Spain (Guadalajara, Castilla-La Mancha), placed in the facilities of the apicultural and agro-environmental research center located in Marchamalo (CIAPA, IRIAF). The material was collected throughout the year 2021 and were kept frozen for analysis. The by-products were crushed to homogenize the residue and set it ready for analysis. Among the parameters analyzed, dry weight, organic matter, water activity, pH, electric conductivity, and N content, it is noteworthy the high C/N ratio obtained. Results were compared with previous results of pollen, and honey samples in order to establish the contribution of these elements to final composition of these by-products. Further analyses are in progress to fully characterize the by-product.





### PP-392

### Physical, chemical and palynological characteristics of honey produced for the Cluster Apícola de la Cuenca del Salado, Argentina

María Soledad García Paoloni<sup>1</sup>, Soledad Varela<sup>2</sup>, Cecilia Mouteira<sup>3</sup>, Ana Clara Dedomenici<sup>3</sup>, Cecilia Alberto<sup>3</sup>, Matias Toledo<sup>4</sup>

<sup>1</sup>- Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Hilario Ascasubi, Argentina

<sup>2</sup>Facultad de Ciencias Agrarias - UNMdP - Área Ciencia de los Alimentos, Balcarce, Argentina

<sup>3</sup>Ministerio de Desarrollo Agrario de la Provincia de Buenos Aires. Dirección Apícola. Laboratorio Central de Apicultura, La Plata, Argentina

<sup>4</sup>Cluster Apícola de la Cuenca del Salado, Argentina

The civil organization "Cluster Apícola de la Cuenca del Salado" (CACS) was constituted in 2017 by beekeeping associations located in the east of Buenos Aires province, Argentina. This territory is immersed in the Pampean Phytogeographic Region; characterized by plains, a temperate to warm climate and rainfall all year round which favours the growth of natural pastures, reason why it concentrates the largest number of beehives in the country. CACS is formed for almost one hundred beekeepers who adhered to a production protocol agreed with INTA-PROAPI technicians to obtain quality honey. To know physical, chemical and palynological aspects of the produced honey, 46 samples were collected from the districts of Rauch (12), Tandil (5), Mar del Plata (5), General Alvear (9), Villa Gesell (9) and Chascomús (6). The sampling was carried out by technical personnel under a procedural protocol during February/March 2021. The physico-chemical parameters studied were colour, moisture/ ash/ hydroxymethyl furfural (HMF) content, pH, free acidity, and conductivity (IRAM techniques). Concerning the first one, 58% of the samples were classified as "light honey" (< 48 mm Pfund) and the conductivity values were those characteristics of "flower honey" according to the Codex Alimentarius. Regarding the pH, most of the values ranged from 3.7 to 3.9. Concerning the rest of the parameter values, all the samples comply with the regulations established by the "Mercosur Technical Regulation for Identity and Quality of Honey". Moreover, the values obtained for acidity, HMF and ash content were much lower than the last mentioned regulation limits. According to the palynological results, 86% of the samples were classified as monofloral, with 69% corresponding to Lotus sp. and 19% to clovers (Lotus sp., Trifolium sp., Melilotus sp., Medicago sativa) which was consistent with the physico-chemical characteristics, especially the predominance of light colours. The remaining monofloral samples correspond to Helianthus sp. (2%), Eucalyptus sp. (2%) and Rhamnaceae (4%). The latter comes from a coastal area, called by beekeepers "coronillo" honey (*Scutia buxifolia*), with very particular physical/chemical and sensory characteristics. Based on these results, it would be possible to produce characterized monofloral honey to add commercial value in the studied area.

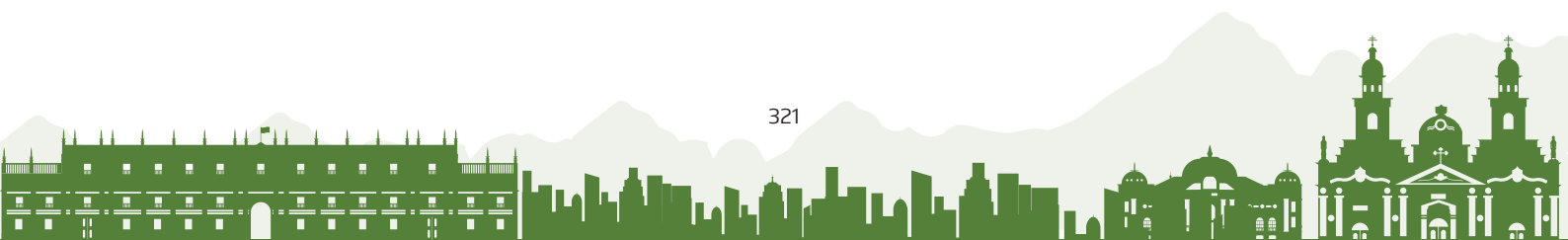
### PP-393

### Chilean Honeys and their functional properties

Gloria Montenegro, Rodrigo Figueroa, Gabriela Cordovez, Gabriel Núñez

Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile.

Chile is one of the world mediterranean climate hotspots and from their endemic and native angiosperms could produce a variety of honeys with functional properties very unique, able to compete today with the most emblematic honeys of the world. In this work we characterized the main types of unifloral Chilean honeys according to the organoleptic, sensorial and antimicrobial activity identifying the geographical area of production. The botanical origin of honeys were determined using the melissopalynology methodology and the antimicrobial activity was evaluated throughout the Well Diffusion in agar method against the bacteria *Escherichia coli*, *Staphylococcus aureus* y *Salmonella enterica* sv. Typhi. The colour was established using the scale Pfund. The production of honeys in Chile occurs mainly in two geographical regions, one of these zones belongs to the arid and semiarid mediterranean climate region dominated by the shrubby evergreen sclerophyllous vegetation, the matorral, region able to produce unifloral honeys such as chañar (*Geoffroea decorticans*) litre (*Lithraea caustica*), coronillo (*Escallonia pulverulenta*), corcolén (*Azara petiolaris*), tevo (*Retanilla trinervia*), peumo (*Cryptocarya alba*) y quillay (*Quillaja saponaria*). The second region correspond to the humid mediterranean zone with the Temperate Valdivian Forest as the main vegetation type and the forestry region of Patagonia regions able to produce honeys such as tineo (*Weinmannia trichosperma*), avellano (*Gevuina avellana*), tiaca (*Caldcluvia paniculata*), ulmo (*Eucryphia cordifolia*), guindo santo (*Eucryphia glutinosa*). The antimicrobial activity goes from honeys very active to less active compared with the antibiotics penicillin and streptomycin. We were able to certify most of these antimicrobial activity applying the Active Patagonia Factor, APF factor. Study financed by Regional Proposals FIC (Fondos de Innovación para la Competitividad Regional) and CMPC that allow us to work closely to beekeepers.



### PP-394

### Looking for pollen spectra characterization of unifloral Eucalyptus honeys from Uruguay

Gloria Daners<sup>1</sup>, Eduardo Dellacassa<sup>2</sup>, Lucía Almeida<sup>2</sup>, Laura Fariña<sup>2</sup>

<sup>1</sup>Instituto de Ciencias Geológicas, Facultad de Ciencias, UdelaR, Iguá 4225, 11400-Montevideo, Uruguay

<sup>2</sup>Laboratorio de Biotecnología de Aromas-DQO-CYTAL, Facultad de Química-UdelaR, Gral. Flores 2124, 11800-Montevideo, Uruguay

The first hives arrived in Uruguay at the beginning of the 19th century, settling in the Colonia Department (southeast of the country), where the initial steps were taken in the beekeeping development. Towards the 50s the activity was consolidated, and professionalization begun by the incrementing demand for bee products also focused on export market. Currently, almost 90% of the production is exported, being an activity of great economic relevance for the country, and particularly for the beekeeping sector. Most of this export occurs as honey in bulk without being classified at origin, so marketed without added value. Moreover, as consequence of the country's forestry policy, there are currently high predominance and abundance of artificial Eucalyptus plantations, also used by the beekeeping sector, being Eucalyptus honeys among the higher produced in Uruguay. Thirty-six (36) samples corresponding to 2020-2021 harvests were analyzed, registering 56 pollen types: 29 at species level, 14 at genus and 13 at tribe or family. Twenty-two (22) samples showed Eucalyptus pollen above 90% of, while 11 had between 80 and 90%. The 3 remaining samples presented Eucalyptus pollen in 77, 73 and 70%. Despite that Eucalyptus is overrepresented in pollen spectra, there is no specific legislation in Uruguay regarding the levels required to classify these honeys as unifloral, while in other countries their legislations consider percentages between 70 and 90%. The main accompanying pollen types had been consistent in both harvests so the characterization of Uruguayan Eucalyptus honeys seems feasible. Baccharis sp. and/or Lotus sp. were present in 100% of the samples being the only two (2) pollen types with percentages above 1% in several samples, reaching up to 17 and 22% respectively. Further studies are needed since analysing honey produced from at least 3 consecutive years is recommended for best and more robust pollen spectra characterization. Keep analysing Uruguayan Eucalyptus honeys will follow global trends for characterization of food products, increase knowledge of local honeys, provide a more qualified spectrum in the domestic market allowing to improve the perception of national consumers. It will also look for an added value that benefits the country's beekeeping production chain. Acknowledgements. Project CSIC-VUSP MOD 2 N° 2054 "Valorización de la Miel de Eucalyptus del Uruguay"

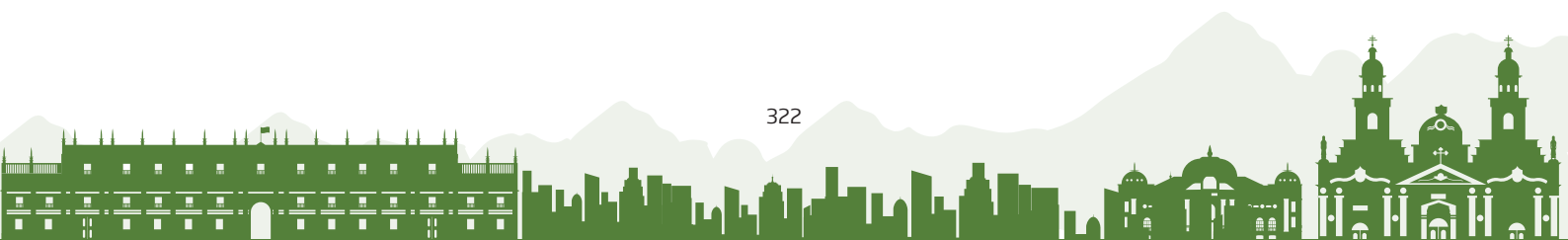
### PP-395

### Evaluation of Near Infrared Spectroscopy for the quantification of quality parameters and botanical origin of honey samples

Mariela Judith Silva, Iris Andrea Lobos

Instituto de Investigaciones Agropecuarias (INIA), Remehue, Osorno, Chile

Honey is a food produced by bees (*Apis mellifera*) from floral nectars, its composition is essentially fructose, glucose, water and to a lesser extent proteins, vitamins, minerals and other components. The honey chemical composition differs by different issues, such as botanical origin, production methods, and storage conditions. At present there are analytical methods that allow us to evaluate these honey issues and determine his quality. These methods are standardized and have quality control. Nevertheless, these techniques have limitations, such as their high cost, they consume a lot of time and, above all, they require highly experienced personnel. The present work proposes to evaluate near infrared spectroscopy (NIRS) as a method of rapid quantification of physicochemical parameters of honey and botanical origin. Honey samples (n=100) were recollected from the Los Lagos Region of Chile, during 2019 season. They were recorded in a BRUKER FT-NIRS MPA equipment to obtain spectra in reflectance mode. Subsequently, the samples were analyzed using the traditional methods, melissopalynological, sugar profile (Glucose, Fructose and Sucrose), HMF, Ash, Protein and Moisture. The predictive models were created using regression by partial least squares (PLS), the performance of the models was determined by the squared correlation coefficient ( $R^2$ ) and the relationship between the standard deviation of the reference chemical method and the prediction error (RPD) of each model, if the value of RPD is greater than 2.5, the model is considered adequate to predict the composition of the different parameters in unknown samples. The main  $R^2$  and RPD results were; Humidity (0.95-4.7), protein (0.72-2.0), Sucrose (0.73-2.9), Fructose (0.54-1.9). Regarding to the pollen prediction, the highlights result were *Eucryphia cordifolia* (0.89-3.2), *Eucalyptus globulus* (0.89-3.16), *Luma apiculata* (0.84-2.5). These data suggest that the NIRS methodology indicates to be a potential tool for quality control in a matrix as complex as honey. However, it is important to continue evaluating models, expanding the range of concentration, include another type of honey and different harvest seasons.



### PP-396

#### Physical-chemical aspects of bee pollen production over 12 months

Denise de Lima Belisario<sup>1</sup>, Lidia Maria Ruv Carelli Barreto<sup>2</sup>, Ricardo De Oliveira Orsi<sup>3</sup>

<sup>1</sup>Universidade de Taubaté - Agronomia - EaD

<sup>2</sup>Universidade de Taubaté - Agronomia - Pós graduação

<sup>3</sup>Universidade Estadual Paulista Júlio de Mesquita Filho

With the objective of evaluating the period of production and the physical chemical profile of the bee pollen produced in the Apiary School of the University of Taubaté, this work systematized daily collections of bee pollen during 12 months through collectors installed in the hives. After collection, the material underwent a series of processes such as: freezing for conservation and sanitization; 4% dehumidification; manual cleaning to eliminate dirt; storage in perfectly sealed clean and dry pots. After processing all the material collected over 12 months, physical and chemical analyzes were carried out at the Laboratory of Quality Control of Apicultural Products at the Center for Apicultural Studies of the University of Taubaté, as well as the capture of images for the formation of the color guide. of the said samples. The indexes for protein were: a minimum of 18.37% in September and a maximum of 26.95% in January; for minerals the minimum was 2.39% in May and a maximum of 3.29% in January and for lipids a minimum of 5.3% in March and a maximum of 7.32% in November and The color guide may possibly suggest 12 types pollen present in the samples over 12 months, reinforcing the efficiency of bee pollen as a natural marker of ecological changes occurring in the studied area, concluding that these results indicate the succession of local flora under the influence of seasonality.

### PP-397

#### Palinological characterization of propoleans of *Apis mellifera* L in the province of Jujuy, Argentina

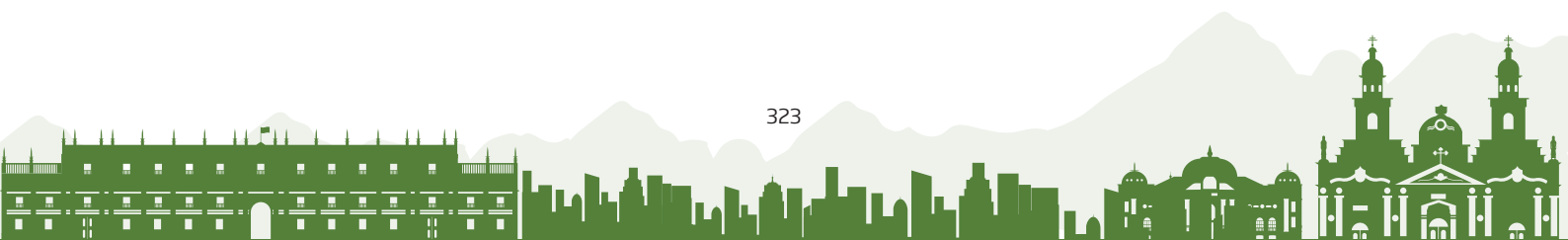
Rosa Milagro Retamoso<sup>1</sup>, Magali Veronica Mendez<sup>2</sup>, Lesli Nicole Vallejos Farias<sup>3</sup>, Gisela Beatriz Ruiz<sup>2</sup>, Marcelo Rafael Benitez Ahrendts<sup>1</sup>

<sup>1</sup>Facultad De Ciencias Agrarias Unju

<sup>2</sup>Inecoa-conicet

<sup>3</sup>Uce Davis Chile

Propolis is a bee product composed of resinous, gummy, balsamic substances, waxes, essential oils and pollen, collected and processed by *A. mellifera* bees. Melissopalynology studies the pollen grains and spores present in bee products and from the presence of some pollen types, the region of origin of the propolis is identified. The objective of the present work was to determine the phytogeographic origin of propolis from beehives in different geographical areas of the province of Jujuy. Para ello, se obtuvieron muestras de propóleos a partir de visitas a los diferentes apiarios situados en el área de estudio (Severino, Tilquiza, Yala, Humahuaca, El Carmen) por el método de mallas colocadas en 5 colmenas de cada apiario. For this purpose, propolis samples were obtained from visits to the different apiaries located in the study area (Severino, Tilquiza, Yala, Humahuaca, El Carmen) by the method of nets placed in 5 hives of each apiary. The phytogeographic origin was determined by means of palynological analysis, which consisted of the recognition and quantification, by microscopic observation, of the pollen grains present in the propolis, allowing the characterization of the vegetation surrounding the hive and also the phytogeographic region. This analysis was carried out at the Palynological Analysis Laboratory of the Faculty of Agricultural Sciences, UNJu. In a total of 30 propolis samples analyzed in the periods and sites studied, a total of 58 pollen types belonging to 34 botanical families were found. Ten were identified at species level, 32 at genus level, 3 at type level, 21 at family level, one at class level and one indeterminate pollen type. The 62% percent belong to native flora, 10.3% to exotic flora and 27.5% to flora of undetermined origin, showing that native resources are the most used. In relation to pollen richness, it was observed that there were no significant differences between the sites studied ( $p=0.89921$ ). From the results obtained, it was determined that the propolis samples represent the vegetation of each site, corresponding to the different phytogeographic zones: Yala and Tilquiza in Yungas, El Carmen and Severino in the Yungas-Chaco transition and Humahuaca in Prepuna.



### PP-398

## Building Comb from Castle to Castle: Collaborations between Queen Breeders & Researchers for supporting Adaptive Reproductive Practices

Melanie M Kirby<sup>1</sup>, Megan Mahoney<sup>2</sup>, Ange Roell<sup>3</sup>, Aiden Wing<sup>4</sup>, Eric & Joy Mcewan<sup>5</sup>, Sam Comfort<sup>6</sup>, Mark Spitzig<sup>7</sup>, Emily Bondor<sup>8</sup>, John Jacob<sup>9</sup>, Tucka Saville<sup>10</sup>, Carl Chesick<sup>11</sup>, Juliana Rangel<sup>12</sup>, Robyn Underwood Margarita Lopez<sup>13</sup>, Judy Wu Smart<sup>14</sup>, Ethel Villalobos<sup>15</sup>, Brock Harpur<sup>16</sup>, Megan Milbraith<sup>17</sup>, Brandon Hopkins<sup>18</sup>, Ellen Topitzhofer<sup>19</sup>, Kaira Wagoner<sup>20</sup>

<sup>1</sup>Adaptive Bee Breeders Alliance, Zia Queenbees Farm & Field Institute

<sup>2</sup>Mahoney's Satellite Selects

<sup>3</sup>New England Queen School, They Keep Bees

<sup>4</sup>Wings of Nature Bees

<sup>5</sup>Diggin' Livin' Apiaries

<sup>6</sup>Anarchy Apiaries

<sup>7</sup>LongeviBees

<sup>8</sup>Santa Cruz Bee Company

<sup>9</sup>Old Sol Apiaries

<sup>10</sup>Tucka Bee, LLC.

<sup>11</sup>Center for Honey Bee Research

<sup>12</sup>Texas A&M University

<sup>13</sup>Penn State University

<sup>14</sup>University of Nebraska-Lincoln

<sup>15</sup>University of Hawaii-Manoa

<sup>16</sup>Purdue University

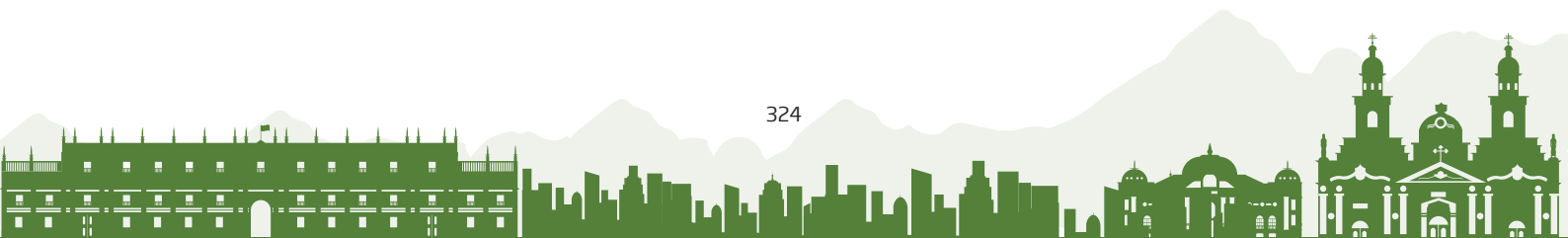
<sup>17</sup>Michigan State University

<sup>18</sup>Washington State University

<sup>19</sup>Cornell University

<sup>20</sup>University of North Carolina- Greensborough

The Adaptive Bee Breeders Alliance (ABBA) is a network of U.S. based bee breeders and researchers working together to support positive pollinator stewardship and healthy food systems through interdisciplinary farm to lab collaborations nurturing adaptive bee breeding methods which mitigate climate change issues through regenerative production practices feeding our biodiverse communities. ABBA is a cross-country projects rooted in 2 previously funded Western Sustainable Agriculture Research Education (WSARE) Farmer grants: The Southwest Survivor Queen bee Project, 2007 and The Rocky Mountain Survivor Queen bee Cooperative, 2012. These bee farmer inspired projects focused on bringing queen honey bee producers together to develop regionally-fortified genetic pools for sharing enduring stock lines. Both projects created networks of beekeepers in diverse communities which helped to raise awareness of distinct bee breeding practices and challenges in the American west. They have inspired the network formation of ABBA to focus on collaborative findings and media guides helping to build bridges between the field and the lab that can help industry and community stakeholders to become better producers and stewards. Building Comb from Castle to Castle includes nuclear genetic stock analysis, Instrumental Insemination training, Cryopreservation of regionally adaptive stock lines, drone congregation areas (DCA) assessments with UAVs, and mentorship and educational outreach opportunities via mixed media offerings to support continued professional growth of both producers and researchers which enhances quality of life and market availability for changing times and climates. This project's goals include promotion of sustainable agriculture by promoting site-specific and regional bee breeding methods that strengthen producers abilities and capacity as necessary for adapting to shifting climate and increasing market demands. This project also enhances the quality of genetics by supporting conscientious production methods. This project began in November 2022 and its establishment will ensure through 2025 with anticipated expansion of services and educational offerings for bee breeders across the Americas and the globe. As stewards, it is imperative that we develop and promote methods and applications that can nurture the next generation of apiculture professionals and the communities they serve.



## Beekeeping Technology and Quality

### PP-399

#### Honey adulteration detection in commercially available honey samples from Panama using HPLC and UHPLC methods

Andrés Rivera-Mondragón<sup>1</sup>, Siara Hernández<sup>1</sup>, Aylin De Lora<sup>1</sup>, Yuliana Christopher<sup>1</sup>, Hermógenes Fernández Marín<sup>2</sup>, Goy Navas Tapiero<sup>1</sup>

<sup>1</sup>Instituto Especializado de Análisis, IEA, Universidad de Panamá, Panamá 0824, Panamá

<sup>2</sup>Centro de Biodiversidad y Descubrimiento de Drogas, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), Panamá 0843-01103, Panamá

Honey is a sweet natural substance produced by diverse bee species and is a foodstuff with great economical value widely used for its nutritional content and health benefits. The quality of honey is threatened by its adulteration through the addition of non-allowed substances that affect its physicochemical, organoleptic, and microbiological properties. The quality of honey is clearly defined and described by international standards such as Codex Alimentarius standard and European Council Directive relating to honey, and laws and regulations implemented at a national level. In recent years there have been great advances in the development of analytical techniques with a higher degree of sensitivity, specificity, and reliability, such as high-performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC) that allow the separation, identification, and quantification of substances present in honey, including sugars, adulterant products, and degradation products such as hydroxymethylfurfural (HMF). This study aims to reveal possible honey adulteration using two HPLC and UHPLC methods. A total of fifty-one commercial honey samples were collected from local markets in Panama and their glucose, fructose, sucrose, and HMF contents were determined using Harmonized Methods of the International Honey Commission (IHC). Overall, we found that 78.4% of samples were suspicious to be adulterated, which means non-compliant with the quality parameters established by the international and local quality standards regarding sugars and HMF content. The HMF contents of 74.5% of the tested samples were beyond the advised standard limits (40 mg/kg), possibly due to overheating and prolonged storage. Additionally, adulteration with high sucrose content was revealed in 17.6 % of samples. These techniques were successful in detecting the percentage of adulterating agents, and they could support the Panamanian authorities by providing a precise, fast, and accurate analytical method for discriminating between authentic and adulterated honey. Adulteration remains an unfair practice for both honest honey producers and consumers.

### PP-400

#### Precise beekeeping development in Ukraine

Tetyana Vasylykivska<sup>1</sup>, Anatoliy Kharkovenko<sup>2</sup>, Borys Shynkaretskyi<sup>3</sup>

<sup>1</sup>NGO "Brotherhood of Ukrainian Beekeepers", Lviv, Ukraine

<sup>2</sup>Association "Professional Pollination of Agricultural Crops", Kyiv, Ukraine

<sup>3</sup>Mobile Soft LLC, Kyiv, Ukraine

Ukraine takes its place in TOP-10 of world grain-producing and TOP-5 of world honey-producing countries. Majority of leading agricultural companies and holdings are integrating precision farming technologies. It is these technologies that allow them to obtain high yields of agricultural products, optimize costs, reduce the negative impact on the environment, and obtain higher-quality products.

Overall production fund of the beekeeping industry of Ukraine is more than 3.6 million bee colonies. Few bee colony smart monitoring systems had been developed in Ukraine for last 20 years. They were quite simple systems with basic features at the beginning. Like simple «weight over GSM» devices. But for today we can rely on real smart systems with rich set of features:

- Bee colony internal parameters (realtime weight, temperature, relative humidity inside beehive, sound spectrum analysis, and flight activity) and external environmental parameters
- Integrated safety and security functions (hive opening and movement notification)
- Robust data acquiring, storage, processing, and visualization on smartphone and PC
- AI data analyzing for warnings, predictions and advising on beekeeper specific actions necessity

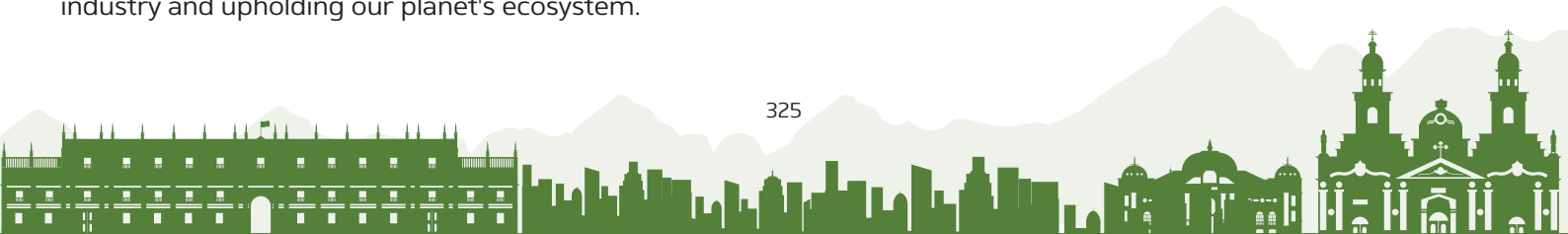
Year by year Ukrainian beekeepers become more familiar with these systems. More and more often you can meet them everywhere from hobbyist to professionals and scientists.

It was the Bee Data system that provided opportunities for private researchers to:

- Studying and exact measuring of bee pollination input to yield of commercial crops of winter rapeseed and high-oleic sunflower
- Studying of wintering of "bank of bee queens" located in usual beehive, standing outdoors
- Studying the economic feasibility of using doubled colonies on the major honey flows

BeeData system is successfully used for monitoring of bee colonies in urban territory for more than two years, providing invaluable assistance and eliminating some specific risks.

Development of smart monitoring systems and their integration into beekeeping processes can play a leading role in improving the health and productivity of bee colonies. This is important for both the sustainability of the beekeeping industry and upholding our planet's ecosystem.





### PP-401 Improving the Danish honey quality

Ole Kilpinen, Flemming Vejsnæs  
Danish Beekeeper Association

The Danish honey market has been under heavy pressure in recent years with low prices and difficult whole sales. Around half the honey sold in Denmark is imported and a major part of this is cheap honey segment. This has led to increasing direct sale from beekeepers to consumers. As a beekeeper association we want to give our members the best opportunities to market their honey products as high quality and at the same time stimulate them to increase focus on honey quality. For that reason, we have developed our own honey laboratory offering fast, cheap honey analysis to our association members. We also introduce a quality label with stronger requirements than the EU honey directive to water content, HMF and diastase activity. We hope that this quality label can facilitate marketing Danish honey as higher quality than most imported honey and also inspire more beekeepers to shift the balance from quantity to more quality in their honey production. In this talk we present an average Danish honey on the parameters of water content, water activity, HMF, conductivity and sugar content. We also discuss the new Danish standard compared to the EU honey regulation standard.

### PP-402 Bottom-up approach for identifying priorities in EU beekeeping concerning Good Practices and Biosecurity Measures

Filippo Jannoni Sebastianini<sup>1</sup>, Lotta Fabricius Kristiansen<sup>2</sup>, Noa Simon Delso<sup>3</sup>, Flemming Vejsnæs<sup>4</sup>, Peter Kozmus<sup>5</sup>, Aránzazu Meana<sup>6</sup>, Leonidas Charistos<sup>7</sup>, Ivana Tlak Gajger<sup>8</sup>, Aleksandra Splitt<sup>9</sup>, Juraj Toporčák<sup>10</sup>, Ellen Danneels<sup>11</sup>, Kirsten Traynor<sup>12</sup>, Aleksejs Zacepins<sup>13</sup>, Gyula Kasza<sup>14</sup>, Robert Brodschneider<sup>15</sup>, Giovanni Formato<sup>16</sup>

<sup>1</sup>Apimondia, Rome, Italy

<sup>2</sup>The Swedish University of Agricultural Sciences, Sweden, Skara

<sup>3</sup>BeeLife European Beekeeping Coordination, Bruxelles, Belgium

<sup>4</sup>Danish Beekeepers Association, Sjælland, Denmark

<sup>5</sup>Slovenian Beekeepers' Association, Lukovika, Slovenia

<sup>6</sup>Universidad Complutense de Madrid, Sanidad Animal, Madrid, Spain

<sup>7</sup>Hellenic Institute of Apiculture, Nea Moudania, Greece

<sup>8</sup>Veterinary Medicine University of Zagreb, Zagreb, Croatia

<sup>9</sup>The National Institute of Horticultural Research, Skierniewice, Poland

<sup>10</sup>University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic

<sup>11</sup>Gent University, Gent, Belgium

<sup>12</sup>University of Hohenheim, Stuttgart, Germany

<sup>13</sup>Latvia University of Life Sciences and Technologies, Jelgava, Latvia

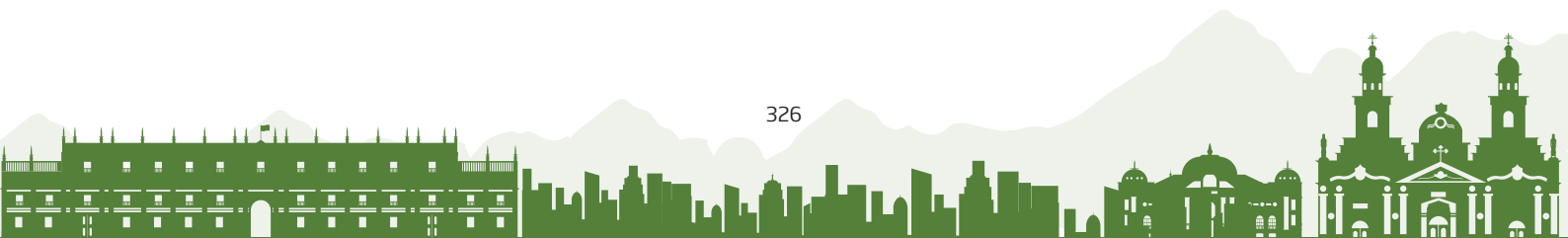
<sup>14</sup>University of Veterinary Medicine Budapest, Budapest, Hungary

<sup>15</sup>University of Graz, Graz, Austria

<sup>16</sup>Istituto Zooprofilattico del Lazio e della Toscana M. Aleandri, Rome, Italy

Good Beekeeping Practices (GBPs) and Biosecurity Measures in Beekeeping (BMBs) play a key role in determining the sustainable management of honey bee colonies. The European project BPRACTICES identified and harmonised a list of GBPs and BMBs encompassing the core aspects of beekeeping management and disease control. A clear view of their practical applicability across different countries is yet needed to provide beekeepers with ready-to-use, practical solutions. The project B-THENET (<https://www.bthenet.eu/>) aims to promote sustainable beekeeping by involving all the main actors of EU beekeeping (beekeepers, advisors, researchers, policymakers, industry, consumers, etc.), in the prioritisation of practices that beekeepers deem most relevant ("priority practices"), and subsequently, their country-level description. We developed a strategy to understand what are "priority practices" for sustainable beekeeping according to EU beekeepers, using a bottom-up approach where practices are rated according to their relevance.

The bottom-up approach to prioritise practices consisted in distributing a survey among EU countries. We asked EU beekeepers to identify what were the most relevant practices according to their opinion, from a list of GBPs and BMBs. To cover all main areas of honey bee management, beekeeping practices were clustered in "themes" to be investigated across the 4 years of the Thematic Network. In 2023, based on their relevance, the following two themes were addressed in the survey: "Apiary set-up and management/maintenance", for the GBPs category, and "Varroosis" for BMBs. In the next years, the following themes will be covered: for GBPs, "Colony set-up and daily management/maintenance", "Record-keeping", "Bee feeding and watering" and "Routine (preventive) hygiene practices"; for BMBs "American foulbrood", "European foulbrood", "Nosemosis, Small Hive Beetle and *Vespa velutina*". We received 2,094 answers from beekeepers in 22 EU countries. The most relevant practices according to beekeepers' needs were: 1. For the GBPs category, practices to "Properly place hives in the apiary" (13 practices); 2. For the BMBs category, practices to "Guarantee best efficacy of anti-varroa treatments" (9 practices). Our results are the first attempt to prioritize, with a bottom-up approach, good practices and biosecurity measures in EU beekeeping.



### PP-403

#### Physical-chemical parameters of melipona honey from Paraíba: uruçú, jandaíra and canudo

Marta Suely Madruga<sup>1</sup>, Jéssica Moreira De Carvalho<sup>1</sup>, Leila Moreira De Carvalho<sup>1</sup>, Fabyan Laís Do Nascimento Guedes<sup>1</sup>, Deyse Alves Pereira<sup>1</sup>, Flávia Izabely Nunes Moreira<sup>1</sup>, Viviane Maria De Sousa Fontes<sup>1</sup>, Gonçalo Pessoa Vicente<sup>2</sup>, Lorena Lucena De Medeiros<sup>3</sup>, Adriana Evangelista Rodrigues<sup>4</sup>

<sup>1</sup>Department of Food Engineering, Federal University of Paraíba, João Pessoa, Paraíba, Brazil

<sup>2</sup>Miel-O, Catolé do Rocha, Paraíba, Brazil

<sup>3</sup>Food Technology Academic Unit, Federal University of Campina Grande, Pombal, Paraíba, Brazil

<sup>4</sup>Department of Animal Science, Federal University of Paraíba, Areia, Brazil

Meliponas produce honey that is different from Apis honey in terms of chemical composition. There is not enough data to establish quality control parameters for these products, mainly due to the lack of research results and the multiplicity of existing stingless bees that produce honey with distinct characteristics. This study aimed to evaluate some physical-chemical parameters that can be used for the characterization and quality control of honey produced by different species of Brazilian stingless bees (*Melipona scutellaris* Latrelle – uruçú, *Melipona subnitida* Ducke – jandaíra, and *Scaptotrigona bipunctata* - canudo). Four different stingless honey samples were collected in the Paraíba State of Brazil in 2023 (Uruçú - U1 and U2, Jandaíra - J and Canudo - C). Honey analyzes, i.e. pH, acidity, moisture, ash, diastase activity and hydroxymethylfurfural-HMF were carried out as described by official methods. The average results for the honeys were varied between: pH 3.20 to 3.93; acidity 12.89 to 73.99 mEq kg<sup>-1</sup>; humidity 21.00 to 25.67 g/100g; ash 0.22 to 0.44 g/100g<sup>-1</sup>; HMF absence at 3.24 mg/kg<sup>-1</sup> and diastase activity 18.18 to 30.08. Honey U1 had higher moisture content and lower pH and ash values, while honey U2 had higher acidity content, lower diastase activity values and no HMF value. Honey J had higher values of pH, HMF, diastase activity and had lower acidity and moisture content, while honey C had higher ash content and did not have HMF, and the high free acid content found for sample U2, with a value of 73.99 mEq kg<sup>-1</sup>. These results reinforce the need for specific regulation for meliponas, since some of the parameters do not meet the existing legislation for Apis honey, with a maximum acidity and humidity of 50 meq.kg<sup>-1</sup> and 20 g.100g<sup>-1</sup>, respectively. This will only be feasible when there is enough data to establish limits for the physicochemical parameters used for quality control. This information reinforces the very peculiar characteristics of meliponas and the need to investigate in more detail the physical and chemical characteristics of honey from different species of stingless bees.

### PP-404

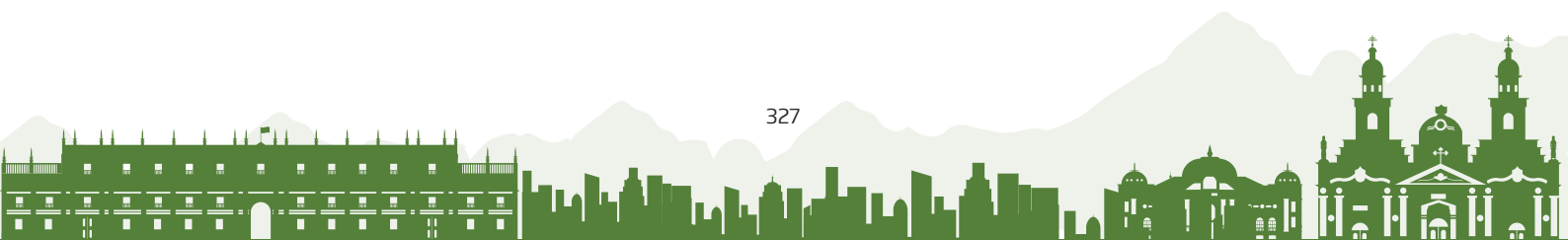
#### Physico-chemical properties of tropical honeys: some particularities that must be taken into account

Carine Massaux, Christine Delcourt, Aurore Dubois, Laurence Leclercq, Béatrice Perat, Virginie Vogels, [Orianne Rollin](#), Etienne Bruneau

CARI, Beekeeping center of research and information, 1348 Louvain-la-Neuve, Belgium

Honey consumers like to have a wide variety of choices. They welcome the fact that tropical honeys with exotic aromas and flavours are presented to them in the market. On the other hand, some countries of the southern hemisphere would like to increase the sale of their honeys in Europe and wish to validate the good quality of these. In this context, the CARI laboratory receives more and more requests for analysis to characterize honeys coming from tropical territories. However, these honeys are composed of nectars and/or honeydew coming from very different plant resources, and harvested according to beekeeping practices adapted to their territory.

We will review some differences between these tropical honeys and our honeys concerning the main physico-chemical parameters: humidity, conductivity, sugar content, HMF (Hydroxy Methyl Furfural), diastase activity and saccharase index. We will also check if the prescriptions of the current European Honey Directive (2001/110/CE of 20/12/2001) and of the Codex Alimentarius are adapted to this type of honey.



### PP-405

#### Characterization of honey produced by species of stingless bees in northeast Brazil

Marta Suely Madruga<sup>1</sup>, Flávia Izabely Nunes Moreira<sup>1</sup>, Viviane Maria De Sousa Fontes<sup>1</sup>, Deyse Alves Pereira<sup>1</sup>, Jéssica Moreira De Carvalho<sup>1</sup>, Leila Moreira De Carvalho<sup>1</sup>, Lorena Lucena De Medeiros<sup>2</sup>, Fabyan Laís Do Nascimento Guedes<sup>1</sup>, Mércia De Sousa Galvão<sup>1</sup>, Octávio Gomes Da Cunha Filho<sup>3</sup>, Adriana Evangelista Rodrigues<sup>3</sup>, José Pereira Da Silva Filho<sup>4</sup>

<sup>1</sup>Department of Food Engineering, Federal University of Paraíba, João Pessoa, Brazil

<sup>2</sup>Food Technology Academic Unit, Federal University of Campina Grande, Pombal, Brazil

<sup>3</sup>Department of Animal Science, Federal University of Paraíba, Areia, Brazil

<sup>4</sup>EMPAER, Catolé do Rocha, Brazil

Stingless bees (SB) produce honeys with excellent nutritional and sensory qualities, which are highly prized for their distinctive flavors. The honeys produced by SB in the Northeast region of Brazil demonstrate the potential of bee flora for the production of honeys with unique characteristics. In view of this, the objective of this work was to carry out a comparative study of the quality aspects of honey produced by four species of stingless bees, *Melipona scutellaris* (uruçu), *Melipona subnitida* (jandaíra), *Scaptotrigona bipunctata* (canudo) and *Cephalotrigona capitata* (mombucão), collected in the states of Paraíba (Caatinga biome) and Bahia (Atlantic Forest biome). Instrumental color ( $L^*$ ,  $a^*$ ,  $b^*$ ), hydroxymethylfurfural, sugar profile and total phenolic compounds were analyzed. The honeys from the Caatinga biome showed higher luminosity (46.59 to 49.06) compared to the honey from the Atlantic Forest (45.68), with the jandaíra honey being the most luminous. In general, the honeys showed a greater tendency towards green coloration ( $-a^*$ ). All honeys showed a predominance of yellow tone ( $+b^*$ ); with emphasis on honey from the Atlantic Forest. The presence of hydroxymethylfurfural was not detected in any of the stingless honeys, thus indicating adequate freshness, handling and storage conditions. Mannose was only detected in uruçu honey (1.04 to 1.48 g/100g), which also had a higher concentration of maltose (0.74 to 1.54 g/100g); while jandaíra honey had the highest concentrations of glucose (42.92 g/100g) and fructose (40.29 g/100g). Honey from the Caatinga biome showed the highest concentrations of total phenolic compounds (43.09 to 63.98 mg CAE/100g), with emphasis on honey from the canudo bee. Given the above, the variation in the composition of the studied honeys can be influenced by a series of factors, such as floral origin, geographic region, type of bee, climatic conditions and harvest time.

### PP-406

#### Popularization of queen rearing: a beekeeping arrangement to maintain a minimal level of production in the persistent context of climate change

Kodjo Logou Agossou

Multiplepurpose Research Production Station, Forestry and Beekeeping Arts Promotion Organization, Guérin-Kouka, Togo

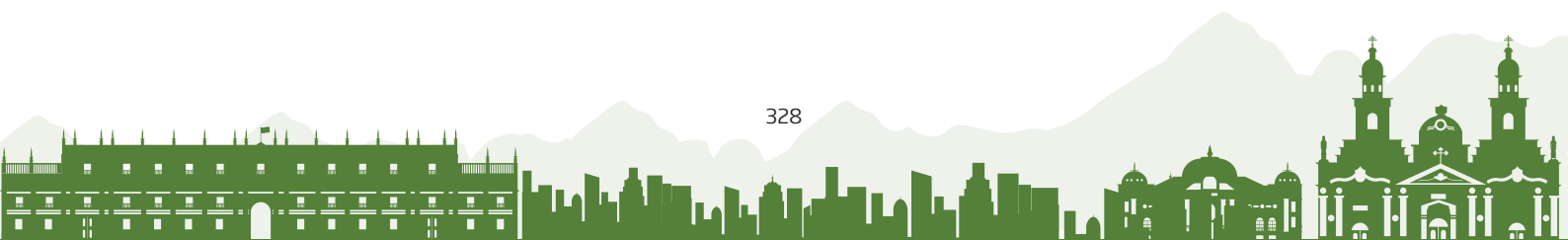
#### POPULARIZATION OF QUEEN REARING: A BEEKEEPING ARRANGEMENT TO MAINTAIN A MINIMAL LEVEL OF PRODUCTION IN THE PERSISTENT CONTEXT OF CLIMATE CHANGE

With climate change, the main manifestations of which are the lengthening or shortening of seasons to which existing production systems and ecotypes of bees and plants are already adapted, queen farming must be the driving force to have the required number and/or size of colonies during the periods when they are most needed.

We must therefore work to ensure that this aspect of beekeeping is well known and widely disseminated.

This should start with some awareness of the standardization of hives suitable for this option. After that, the real work of training in this production technique can begin, which can only be promoted if the equipment is genuinely accessible. It is the place to seek the goodwill of policy-makers as well as that of development institutions for grants and a real program to accompany the sale of queens.

Prior to this project, major work on setting up special apiaries with a genetic bank character resulting from the selection of ecotypes with several adaptive potentials must be created by large ecological zones. It is then necessary to have a fairly exhaustive list of beekeepers in each of these areas. With tact and multidisciplinary professionalism it would now be possible to produce in harmony with the new parameters introduced by climate change while awaiting the results of biodiversity restoration efforts that almost all communities are now aware of



### PP-407

#### Monitoring the quality of honey in Slovakia

Daniela Matisová, Róbert Chlebo

Slovak University of Agriculture, Faculty of Agrobiological Sciences, Tr. A. Hlinku 2, Nitra, Slovakia

The quality of honey from producers and retail chains in Slovakia was monitored during the years from 2013 to 2021. Fructose, glucose, sucrose and water content, electrical conductivity, diastatic activity, HMF, acidity, optical rotation, presence of ammonia sulfite cooler E150d, sensory evaluation and the presence of antibiotic residues were evaluated. In part of honey samples also the enzyme activity of foreign enzymes, B-fructofuranosidase, B/gamma amylase and C3/C4 sugars was determined.

During the mentioned period 692 honeys directly from beekeepers were analyzed of which 50 honey samples did not meet some legislative limits, which represents 7.22%. The analysis of honey from the shops and packaging facilities were performed within the framework of Food surveillance and targeted controls programme of the State Veterinary and Food Administration of the Slovak Republic. In total, 1267 samples of honey were taken, from which 88 samples did not meet the quality criteria, which represents 6.95%.

As nowadays it is not possible to detect the impairment of honey quality with a single analytical method, new approaches of adulteration are discussed.

### PP-408

#### Chemical Analysis, Antiradical And Antimicrobial Activity Of Bee Pollen Collected by "Melipona mandacaia" Smith and "Trigona spinipes" Fabricius

Tania Maria Sarmento Silva<sup>1</sup>, Amanda Monteiro<sup>1</sup>, ayala pereira Pereira Gomes<sup>1</sup>, Camila Macaubas Da Silva<sup>1</sup>, Celso Camara<sup>1</sup>, Francisco Deassisribeiro Santos<sup>3</sup>, Eva Sarmento Da Silva<sup>2</sup>, Yan Lima<sup>4</sup>, Hélder Souza<sup>1</sup>, Mateus Matiuzzi Da Costa<sup>2</sup>

<sup>1</sup>Department of Chemistry, Federal Rural University of Pernambuco, Recife, Brazil.

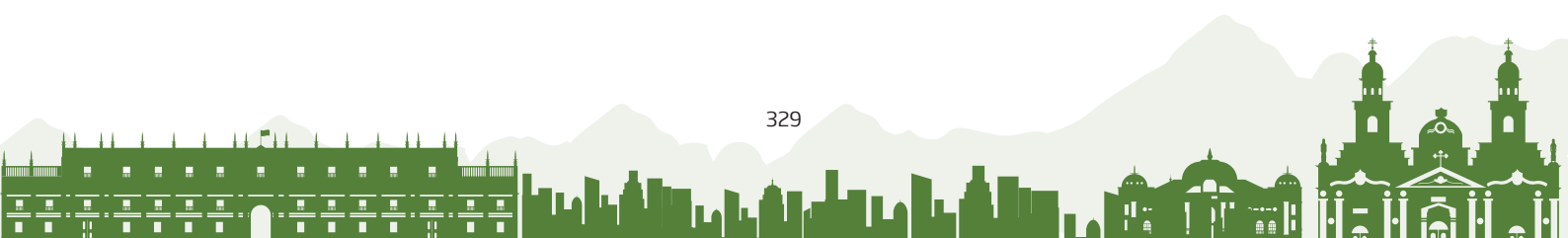
<sup>2</sup>Collegiate of Zootechnics, Federal University of São Francisco Valley, Petrolina, Brazil.

<sup>3</sup>Department of Biological Science, State University of Feira de Santana, Feira de Santana, Brazil.

<sup>4</sup>Paulista State University Julio de Mesquita Filho, Brazil.

The stingless bees are found in tropical and subtropical regions of South and Central America, Africa, Southeast Asia, and Australia. In Brazil they are distributed throughout the country, especially the North and Northeast, where several species are found, among them "Melipona mandacaia" and "Trigona spinipes", are popularly known as mandacaia and arapuá, respectively, being mandacaia created to produce honey and pollen. The objective of this work was to evaluate the chemical composition, antioxidant activity, antimicrobial activity, and palynological analysis of the pollen extracts collected by bees "M. mandacaia" and "T. spinipes". The 11 pollen samples were collected in the states of Bahia and Pernambuco, Brazil. The extracts and fractions were analyzed by ultra-performance liquid chromatography coupled with diode array detector and quadrupole time-of-flight mass spectrometry, it was possible to identify flavonoids such as kaempferol, isorhamnetin, and isoquercitrin, as well as the isolation and identification of, N1, N5, N10 trisynapoyl-spermidine. The palynological analysis of the samples revealed the presence of 15 pollen types for the samples of "Trigona spinipes", with the major component, being "Prosopis juliflora", and 13 types for the samples of "Melipona mandacaia", with "Mimosa tenuiflora" as the dominant pollen type. The samples demonstrated a promising antiradical activity and antibacterial activity against strains of "Staphylococcus aureus" isolated from bovine mastitis. The data contribute with chemical and biological parameters to determine the identity and quality of the bee pollens from "Trigona spinipes" and "Melipona mandacaia", thus contributing to aggregate values to the product.

Acknowledgment: CNPq, CAPES, FACEPE.



### PP-409

#### Estimating particulate matter pollution using honeybees: case study in Dominican Republic

Précillia Cochard<sup>1</sup>, Daniela Castro<sup>2</sup>, Yeray Álvarez<sup>3</sup>, Nicolas Brehm<sup>4</sup>, Benjamin Poirot<sup>1</sup>

<sup>1</sup>APILAB Bureau d'étude environnementale par l'abeille, Lagord, France

<sup>2</sup>NBC Bureau d'étude eau et environnement, Cayenne, French Guiana

<sup>3</sup>TECMALAB Consultora ambiental dominicana, Santo Domingo, Dominican Republic

<sup>4</sup>MELILAB, Estado de México, Mexico

Bees are considered excellent environmental biomonitoring agents because their behavior and activity are closely related to the health and quality of the environment in which they live. As pollinators, bees rely on the supply of flowers and plants, and therefore, are exposed to the quality of air, water, and the presence of contaminants in their environment.

In this context, bees are exposed to multiple pollutants, including particulate matter. Particles can have a natural and/or anthropogenic origin, including trace metals, pesticides, and other toxic substances. Exposure to this pollution can affect the bees' ability to find food and return to the hive, as well as their memory and sense of smell.

In this sense, this study focused on estimating particle pollution using honeybees as bioindicators. To do this, two sites in the Dominican Republic were selected, one industrial-mining and one urban, beehives were installed, and bees were sampled twice a year for a period of three years. The particles present in the bees were analyzed under a scanning electron microscope and were subsequently compared with bees from a reference site.

Bees sampled at both urban and mining sites were found to be more contaminated by PM than those sampled at control sites. Coarse particles were mainly composed of limestone and aluminosilicates from the natural environment. We also found finer particles, often associated with metals. The PM analysis allowed us to relate them to the geochemical and industrial context of the sites.

The results of this study have important implications for human health and the environment, as honeybees can be used as an effective tool for detecting particle pollution in the air. Additionally, these findings can help environmental protection measures to be taken to reduce exposure to pollutants and improve environmental quality.

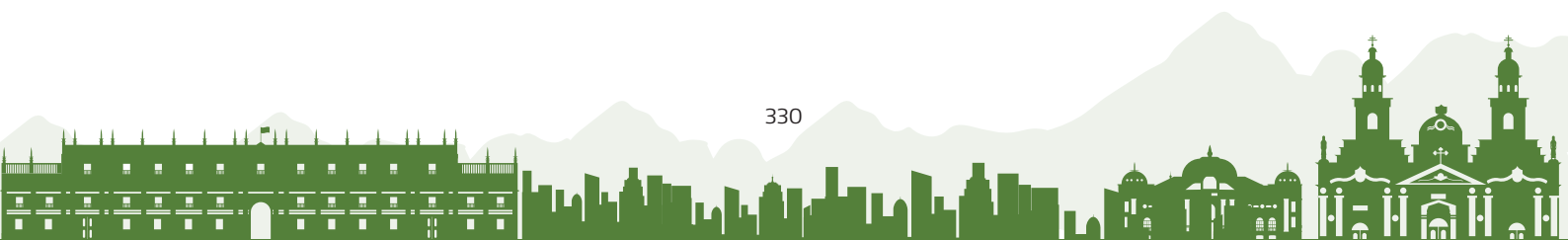
### PP-410

#### The green color of honey produced in Rio Grande do Sul, Brazil, may be due to the addition of natural resin by bees

Karen tanise Da Silva Azevedo, Gilnei Schalavin Viland, Velci Queiróz de Souza, Susiane Cavinatto Meira, Leandro Ademar Lissner, Tanira Da Silveira Prieto, Thays Barboza Da Luz, Andrés Delgado-Cañedo

Programa de pesquisa e extensão em apicultura do Pampa (APIPAMPA), Universidade Federal do Pampa - Campus São Gabriel, São Gabriel, RS, Brasil.

According to the Codex Alimentarius, bees collect nectar, plant secretions, and phytophagous insect excretion, combine them with their own unique substances, and then age the mixture in combs. Several physical and chemical parameters are used to determine honey's integrity. Classification of honey is based on its color, which ranges from light honey, nearly transparent, to its gradient of coloration until it reaches dark brown, nearly black; however, this color is always found within a brown or copper tone, which may be faintly reddish. The market accepts these honeys, whereas honeys with distinct colors may be perceived as having a questionable origin. This may be the case with green and blue honeys, in which the presence of sweet materials from a factory has been confirmed, as well as with green honey from the Philippine island of Palawan, in which it remains unclear whether the coloring is natural or artificial. However, there have been reports of green honey, such as the green honey from Banggi Island in Malaysia, whose green color is due to chlorophyll. There have been reports of the production of green honey in the Brazilian state of Rio Grande do Sul over the past decade, but its origin is unknown. In this study, a physical-chemical analysis of green honey collected in São Gabriel, RS, Brazil, showed comparable physicochemical values to those established by the Codex Alimentarium. The inability to dissolve the green molecule in organic solvents indicates that it is polar and likely not chlorophyll. In our quest for green materials close to the apiary, we discovered a green resin on the trunk of a tree known in Brazil as "Aroeira salsa ou Anacauita" (*Schinus molle*). We propose this resin as a possible source of the molecules responsible for this coloring. We are currently awaiting data from mass spectrometry to verify our hypothesis, which could expand the definition of honey to include the fact that bees deposit resins in this bee product.





## Beekeeping Technology and Quality

### PP-411

### Queen and bee packages production outside of the natural floral availability time in Central Chile

Pablo Astorga Roman<sup>1</sup>, Joaquín Núñez<sup>2</sup>, Jorge Pérez<sup>3</sup>, Juan Velozo<sup>4</sup>, Alexis Vega<sup>4</sup>, Ariel Rojas<sup>4</sup>

<sup>1</sup>Campos de Abejas SpA

<sup>2</sup>Huilmay Bee SpA

<sup>3</sup>Apícola Raytrai Ltd

<sup>4</sup>Universidad Mayor, Facultad de Ciencias, Ingeniería y Tecnología. Centro Tecnológico de Recursos Vegetales

Fifteen years of continuous drought and raising temperatures in Central Chile, related to climate change, had negatively impacted apiculture, producing a progressive shortage of floral resources for bees' foraging, diminishing honey production and profits. To improve apiculture sustainability under this scenario, and given a non-satisfied demand of foreign markets for queens and bee packages, the Universidad Mayor has carried out in Central Chile several projects about foraging bees using buckwheat as a multipurpose crop. This zone has a Mediterranean climate, with 6-8 dry months, where the buckwheat crop requires irrigation and only a small input of fertilizers. Buckwheat sowing can be scheduled as to have a continuous supply of nectar and pollen throughout summer months, until the onset of autumn (4 extra flowering months per season). Even a single sowing could extend the bees' forage availability for another 45 days, improving the yield of biological products, sometimes doubling the spring production and lowering the need for supplemental feeding. Also, healthier colonies are obtained. Two examples at commercial level are given (results obtained by apiculturists associated to the projects). 1) Queen production. Five successive sowing dates, covering from late spring to late summer were set up as to have a continuous availability of bees' forage. The flowering time of each sowing date overlap the previous one in some degree. Surface area of each sowing was about 4.000 m<sup>2</sup> (0,98 acre). Fifteen day after the first sowing, 100 baby nuclei were established in one of the field borders. At the end of this crop, a 100 new baby nucleus were produced, totalizing at the end of season 600 new baby nuclei, plus 300 brood and 300 food frames. The final product was 600 queens for export market. 2) Bee package production. One hectare (2 ac.) was sowing with buckwheat seeds at the end of spring. Full flowering was reached at the beginning of summer, time when 100 nuclei were taken to that field. This flowering was out of season and in one month, produced 100 more nuclei. Details are presented in the poster. We thank the FIC IDI 40017929-0 Grant.

### PP-412

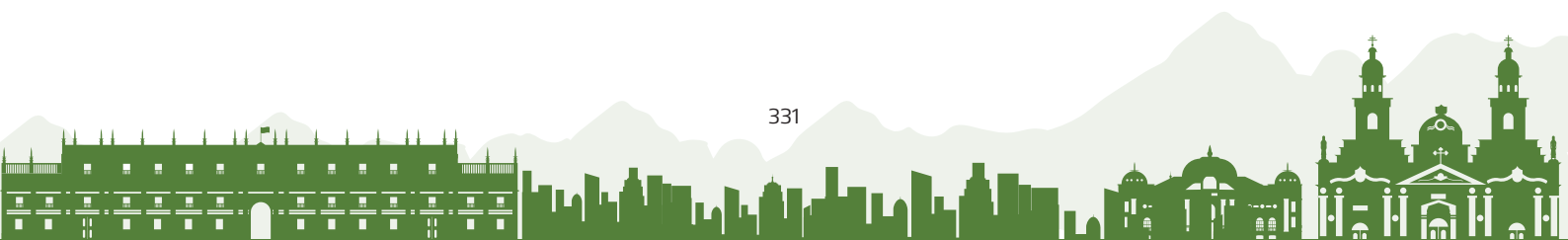
### Evaluation of physicochemical parameters of honey samples produced in the Brazilian Pampa Biome

Leandro Ademar Lissner, Milena Piaia Barboza, Guilherme Henrique Aparecido De Oliveira, Eduardo Guilherme Molero Dos Santos, Flávia Santos, Nicolly Maria Payva Nunes, Marcos Rocha Grillo, Andrés Delgado-Cañedo

Programa de pesquisa e extensão em apicultura do Pampa (APIPAMPA), Universidade Federal do Pampa - Campus São Gabriel, São Gabriel, RS, Brasil.

Historically, the Bioma Pampa region of Rio Grande do Sul, Brazil, has been the Brazil's largest honey producer; however, this honey is typically distributed in bulk without identifying its floral source, and physicochemical data on these are difficult to obtain. Our research group is working on a project titled "Pampa's Honey" in an effort to characterize these products and increase their added value. In this initiative, we are analyzing the physicochemical characteristics of Pampa Biome samples of *Glycina max*, *Eucalyptus saligna*, *Eucalyptus sp*, *Myracrodruon urundeuva*, *Schinus polygamus*, *Echium plantagineum*, and multifloral honeys. Our moisture analysis revealed that the honeys ranged between 18% and 22.50%, and, with the exception of two samples, all the honeys met the maximum limit established by Brazilian law ( $\leq 20\%$ ); the acidity of all the honeys was within acceptable parameters, between 23.18 MEqKg and 27.26 MEqKg ( $\leq 50$  MEqKg). The pH of the majority of honeys was between 3.76 and 4.0, remaining within the acceptable range ( $\leq 4.0$ ). However, the pH of some *Eucalyptus saligna* honey samples was slightly higher than expected, at 4.2%. The conductivity test revealed an average conductivity of  $253.5 \pm 86.6 \mu\text{S}$ , which was within the expected range for all samples ( $\leq 800 \mu\text{S}$ ), with eucalyptus samples exhibiting the highest conductivity values. None of the honeys examined exceeded acceptable levels of reducing sugars or ash. Analyses of diastase activity, apparent sucrose, and insoluble solids are currently being conducted, and analyses of HMF, antioxidant capacity, flavonoid concentrations, and total phenols will be conducted subsequently.

In conclusion, we were able to observe the physical and chemical characteristics of some of the honeys produced in the Brazilian Pampa region, which, with a few exceptions, exhibited the quality expected of honeys produced in accordance with good manufacturing practices. The goals of this project are to increase the number of samples analyzed over a two-year period, share the results with the partner beekeepers, and advise them so that their honeys always meet the physical-chemical parameters stipulated by the regulations.



### PP-413

#### The internal supply of water as a maintenance strategy for apiaries in semi-arid regions

Juliana Nascimento Bendini, Maria Mayara Vieira

Federal University of Piauí, Teresina, Brazil

The semi-arid climatic regime is marked by high temperatures, low rainfall and humidity, which makes the maintenance of bee colonies a great challenge for beekeepers, especially during the dry season. Thus, the objective was to evaluate the internal supply of water in the development of Africanized bee colonies during the dry period in the semi-arid region. The research was carried out in an apiary located in a dry forest area in the municipality of Vera Mendes/Piauí, during the months of September and November 2020. For this purpose, 9 hives were selected based on population equity and food stock (honey and pollen). Before starting the research, the zootechnical form was reviewed and filled in with data regarding the number of brood and food frames and the count of the brood area (1cm<sup>2</sup>) every 21 days. Boardman-type feeders were installed on the hives' surfaces, following the corresponding division of the hives into different treatments: T1- three hives received abundant water (containers filled up to 500 mL), T2- three hives received restrictive water (containers filled with 74.6 mL/day) and T3- three hives did not receive water in the hive, only from external water sources 400 meters away from the apiary. Digital thermo-hygrometers were installed between the central frames of each hive and on the outside of the apiary to measure internal and external humidity and temperatures. Data were analyzed and tabulated and submitted to descriptive statistics and Analysis of Variance using the F test ( $p < 0.05$ ), with comparison of means using the Tukey Test ( $p < 0.05$ ). It was observed that the internal supply of water did not influence the bioclimatic parameters (temperature and humidity) of the colonies. However, the hives that received greater amounts of water had an increase in their pollen storage and consequent increase in breeding areas. Thus, the internal supply of water for bees is recommended as a maintenance strategy for *Apis mellifera* colonies during the dry period in semi-arid regions.

### PP-414

#### Green propolis from the northeastern Brazilian Caatinga plant *Mimosa tenuiflora* (Fabaceae): influence of sunlight and shade in the semi-arid region of Rio Grande do Norte

Jessica Aparecida De Lima<sup>1</sup>, Tuanny Daniele De Araújo Gomes<sup>2</sup>, Katia Peres Gramacho<sup>2</sup>, David De Jong<sup>3</sup>, Andresa Aparecida Berretta<sup>1</sup>

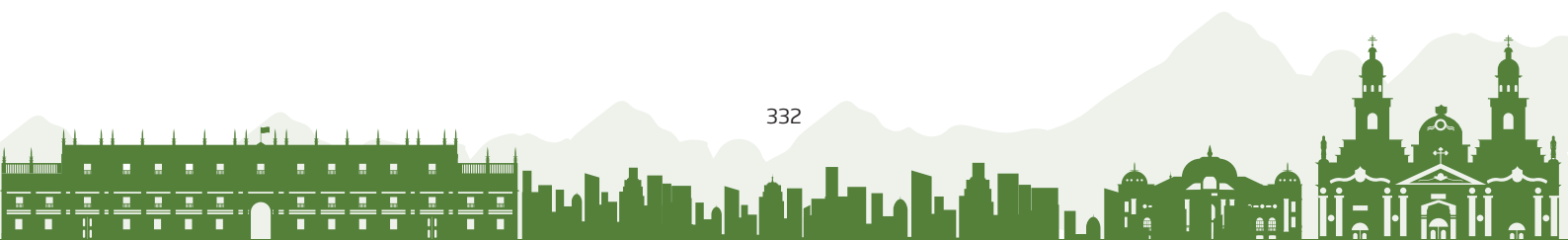
<sup>1</sup>Research, Development and Innovation Laboratory, Apis Flora Industrial e Comercial Ltda, Ribeirão Preto/SP, Brazil

<sup>2</sup>Department of Animal Science, Rural Federal University of Semi-Árido, Mossoró/RN, Brazil

<sup>3</sup>Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, SP, Brazil.

**INTRODUCTION:** Propolis is produced by honey bees (*Apis mellifera*) from plant parts and resins that they collect to defend the colony from pathogens and intruders and help maintain the hive temperature and humidity. Caatinga green propolis is produced by bees from fragments of plant buds of *Mimosa tenuiflora*, locally known as "jurema-preta" (black jurema), mixed with beeswax. This plant is widely distributed throughout the northeastern Brazilian Caatinga region. Shading beehives can help minimize heat stress suffered by bees in these hot semi-arid areas. The objective of this study was to evaluate the influence of different thermal conditions in shaded and unshaded apiaries on the production and quality of propolis produced in honey bee colonies.

**METHODOLOGY:** The experiments were performed with colonies of Africanized honey bees, maintained in sunlight or shade, in the state of Rio Grande do Norte, Brazil. Temperature and humidity within the hives were measured. Propolis samples were collected from the colonies, and then submitted to ethanolic extraction in a Soxhlet apparatus. Total phenolics and flavonoids were evaluated in the extracts. Analysis was made with Sidak-Bonferroni multiple comparisons and unpaired t test with a significance level of 0.05. Propolis production was also compared. **RESULTS:** The percentages of total phenolics ( $10.13 \pm 0.6$  and  $12.71 \pm 0.7$ ) and flavonoids ( $6.618 \pm 0.9$  and  $9.713 \pm 0.7$ ) in the propolis samples from hives maintained exposed to the sun or shade, respectively. They were significantly higher in the propolis collected from the hives maintained in the shade. Propolis production was also significantly higher in hives kept in the shade. The colonies located in the shade had higher humidity and lower temperatures than those in the sun. **CONCLUSIONS:** Shading resulted in better thermal comfort for the bees and greater development of the colonies. Consequently, hives maintained in this environmental condition produced more and better quality propolis.



### PP-415

### Use of near infrared for the classification of honey according to its glyphosate content

Laura Fariña<sup>1</sup>, Gloria Daners<sup>2</sup>, Ana Bonini<sup>1</sup>, Ana Godoy<sup>1</sup>, Lucia Almeida<sup>1</sup>, Eduardo Dellacassa<sup>3</sup>, Eduardo Boido<sup>1</sup>

<sup>1</sup>Área de Enología y Biotecnología de las Fermentaciones, Facultad de Química, UdelaR, Montevideo, Uruguay

<sup>2</sup>Instituto de Ciencias Geológicas, Facultad de Ciencias, UdelaR, Montevideo, Uruguay

<sup>3</sup>Laboratorio de Biotecnología de Aromas, Facultad de Química, UdelaR, Montevideo, Uruguay

The presence of glyphosate in honey has become a problem in countries where this type of herbicide is used to control weeds. The European Union sets a maximum residue limit of 50 ppb glyphosate in honey.

Near infrared spectroscopy (NIRS) is widely used as a rapid method for food analysis. The main features of this technique are its rapidity of measurement, the possibility to quantify several parameters with a single analysis, its high sensitivity, the low cost per analysis and its environmental friendliness (no reagents or solvents are used).

In a NIRS calibration, the spectral information is related, by means of an algorithm, to the physico-chemical composition information (reference method) through the application of statistical models. The aim of this work was to develop a rapid and cost-effective analytical methodology to classify honeys on the basis of glyphosate content.

The original samples used for model development were 5 honey samples contaminated with different levels of glyphosate, thus generating 75 samples. In order to obtain the best calibration model, different pre-treatments of the data were tested and a set of samples was used for validation, obtaining the best parameters in the calibration with data smoothing by 3-point averaging and subsequent second derivative of the spectrum by the Savitzky-Golay method. The calibration obtained in the model accurately predicted the glyphosate content in 80% of the honey samples of the prediction batch. In a second step, 44 samples were added to the calibration model, all of them with different pollen origins. Another 10 honeys were measured together to provide a set of external samples to the model. The calibration obtained on this occasion accurately predicted the glyphosate content in 70% of the honey samples, using 50 ppm glyphosate as the cut-off point. This model could be improved by performing specific calibrations for honeys of a defined geographical or pollen origin to reduce the matrix effect.

### PP-416

### The Geographical Indication Lemon Blossom Honey from Tucuman (Argentina) as a value-added strategy

Alejandro Álvarez<sup>1</sup>, Gabriela Marcello<sup>2</sup>, Javier González<sup>3</sup>, Virginia Salomón<sup>1</sup>, Carina Sánchez<sup>4</sup>, Liliana Lupo<sup>4</sup>, Verónica Albarracín<sup>5</sup>, María Cavallera<sup>6</sup>, Enrique Bedascarrasbure<sup>7</sup>, Luis Maldonado<sup>1</sup>

<sup>1</sup>National Institute of Agricultural Technology INTA EEA Famaillá – PROAPI – Ruta Prov. 301 km 32 – Tucuman – Argentine

<sup>2</sup>Ministry of Production of Tucumán-Food Directorate– Tucuman – Argentine

<sup>3</sup>Norte Grande Cooperative Ltda., Ruta 301 km 18 – Lules – Tucuman-Argentine.

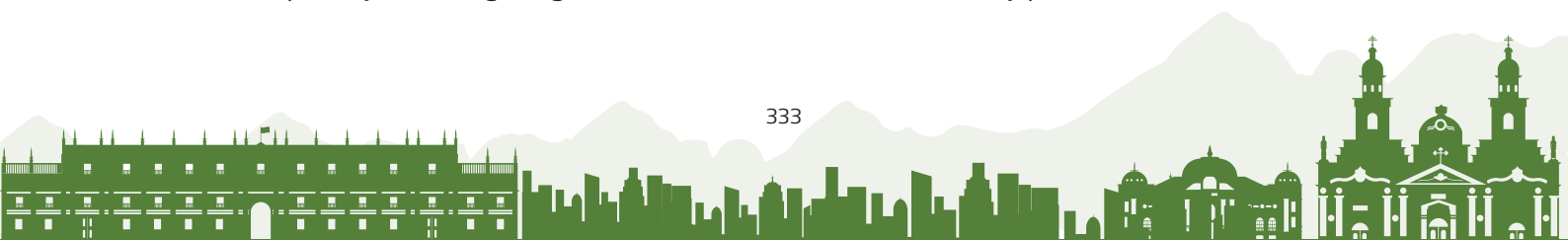
<sup>4</sup>National University of Jujuy-INECOA (CONICET)-Palynology Laboratory – Tucuman-Argentine.

<sup>5</sup>National University of Tucumán-FAZyV – Tucuman-Argentine.

<sup>6</sup>Secretary of Agriculture, Livestock and Fisheries from Argentine- Northwest Focal Point– Tucuman-Argentine.

<sup>7</sup>Research Center of Agroindustry INTA CIA – PROAPI - Repetto y de los Reseros- Castelar-Buenos Aires –Argentine.

A Geographical Indication (GI) is a value-added tool that allows identifying the differential characteristics of a product, associating them with the territory that gives it origin, and with the practices that producers develop to obtain it. The objective searched in this work is to achieve the recognition of the GI of Lemon Blossom Honey from Tucuman, in order to increase the income of small associated beekeeping producers through the valorization of a typical product from the province of Tucuman, Argentina. To achieve this objective, the physico-chemical, sensory, melissopalynological and functional characterization of lemon honey from Tucuman was carried out. Among the analytical parameters determined, it is worth highlighting the hesperidin content, which gives functional properties to lemon blossom honey, such as its antioxidant and phlebotonic properties. This flavonoid (hesperidin) also is a marker of floral origin. A Protocol of Good Management and Manufacturing Practices was developed and implemented to obtain monofloral lemon honey in a consensual manner with the Norte Grande Cooperative and ACTA (Association of Beekeeping from Tucuman). In addition, an interinstitutional organization for the recognition of the Geographical Indication Blossom Lemon Honey from Tucuman was formed, made up of the Food and Livestock Directorates of the province of Tucuman, the National University of Tucumán, the Argentine Northwest Focal Point of the Secretary of Agriculture, Livestock and Fisheries from Argentine, INTA (National Institute of Agricultural Technology) and the beekeeping organizations. Workshops and surveys were carried out with producers and consumers to verify their recognition of the differential sensory characteristics of lemon blossom honey from Tucuman. Compliance audits of the lemon blossom honey production protocol were carried out. Based on these activities, the GI recognition request form was prepared, which is in the process of being approved by the Secretary of Agriculture, Livestock and Fisheries of Argentine. We believe that this differentiation and added value tool will improve the income of small and medium associated beekeepers by achieving a higher sales value for their lemon honey production.



### PP-417

#### Assessment of *Quercus pyrenaica* honeydew honey origin as a tool for its valorisation: the perspective of the ACORNDEW Project

Soraia I. Falcão<sup>1</sup>, Kheira M. Mouffok<sup>1</sup>, M. Shantal Rodriguez Flores<sup>2</sup>, Olga Escuredo<sup>2</sup>, M. Carmen Seijo<sup>2</sup>, M. Eliza Sousa<sup>1</sup>, Albino Bento<sup>2</sup>, António Castro Ribeiro<sup>2</sup>, Antero Sousa<sup>3</sup>, Ana Tomás<sup>3</sup>, Joana Amaral Paulo<sup>4</sup>, Miguel Vilas Boas<sup>1</sup>

<sup>1</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

<sup>2</sup>Department of Plant Biology and Soil Sciences, University of Vigo, Campus As Lagoas, 32004 Ourense, Spain

<sup>3</sup>Bota um Cibo, Prada, 5320-221 Vila Verde, Vinhais, Portugal

<sup>4</sup>Forest Research Centre (CEF), School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal

Honey can be produced by honey bees by collecting the nectar of flowers, and classified as nectar honey, or from plant secretions or excretions of plant-sucking insects on the living parts of plant as they feed on plant sap and classified as honeydew honey. Honeydew honeys are darker than most nectar honeys and usually described as having higher values in several quality physicochemical parameters. The commercial interest in honeydew honeys is growing due to its higher therapeutic properties when compared to nectar honeys, but also due to its strong flavor and mineral richness. To avoid adulterations and frauds, and to enhance its market value, it is fundamental to find reliable parameters for authentication of this honeydew honeys and link them to the botanical source. The Pyrenean oak or black oak (*Quercus pyrenaica*), due to their extension, continuity, and conservation status, is considered the most representative tree species in the Montesinho Natural Park (MNP) and one of the most significant Pyrenean oak forests in Europe. Oak honeydew production from plant secretion was reported to be the main botanical source of honeydew honey in the Iberian Peninsula, but nothing is known about insect contribution or the effective productivity of sweet saps from *Q. pyrenaica*. Within the ACORNDEW project, and to fulfil the objectives, honeydew honey production is being assessed through the management of 4 apiaries established in MNP black oak areas located at different heights, where the meteorological data is being monitored as well as the honey production. At the same time, the insects living in oaks were collected, identified, their life cycle studied, and the oak sap production was being monitored and characterized. The chemical composition and melissopalynological analysis of *Q. pyrenaica* sweet saps and honeydew honey was been characterize as a tool for the botanical origin authentication.

### PP-418

#### Advances towards a bee packages maritime transport

Alexis Vega<sup>1</sup>, Juan Velozo<sup>1</sup>, Ariel Rojas<sup>1</sup>, Isabel Del Real<sup>1</sup>, Joaquín Núñez<sup>2</sup>, Patricio Sáez<sup>3</sup>, Patricia Aldea<sup>4</sup>

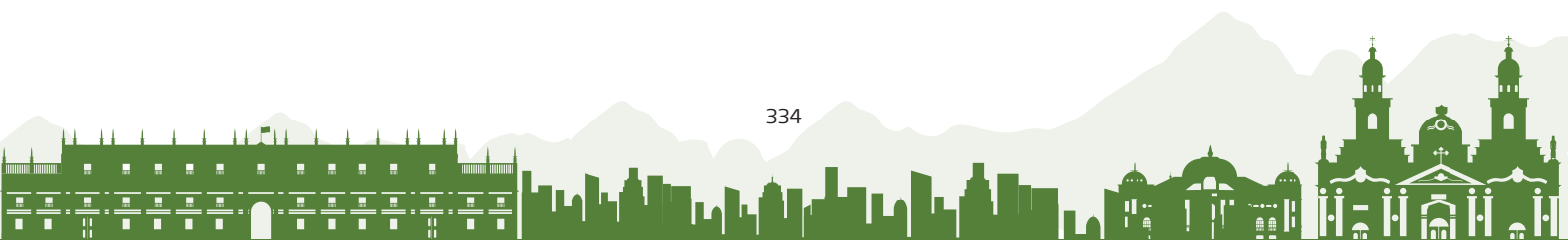
<sup>1</sup>Universidad Mayor, Facultad de Ciencias, Ingeniería y Tecnología. Centro Tecnológico de Recursos Vegetales

<sup>2</sup>Huilmay Bee SpA

<sup>3</sup>JPM Exportaciones

<sup>4</sup>Universidad SEK, Instituto de Investigación Interdisciplinar en Ciencias Biomédicas SEK

Climate change is affecting beekeeping activity in Chile. In the Chilean Central Zone, with a Mediterranean climate moving towards an arid climate, most of the bees' native floral resources have been not able to withstand the drop in rain average from 500 to 150 mm yearly, affecting commercial apiaries by diminishing honey production/hive from 50 to 12 Kg, which is not commercially sound. Alternative products are queens and bee packages for export, with better profit and advantages under the current ambient conditions. However, air transport has been problematic and it is volume limited. To solve this logistic problem, the Universidad Mayor has been studying the feasibility of a bee package maritime transport, using controlled atmosphere containers. Results can be summarized as follow for a 30-35 days period of transport: a) Bees do not require previous conditioning for cold storage, neither to get out of it. b) Bees inside a cardboard box under cool storage showed circadian cycles, which can be sensed registering box temperature, relative humidity and CO<sub>2</sub> concentration. Temperature and CO<sub>2</sub> concentration showed inverse dynamics compared to relative humidity. Any stress on bees can be detected through anomalous circadian patterns. c) Under cold storage, bees' survival critical factors are feedstuff amount and composition, CO<sub>2</sub> concentration control, temperature and relative humidity settings. d) Ambient temperature determines feedstuff consumption and if it is equal or bigger than 8°C, the consumption rate can be excessive, shortening the cold storage period. For a 1 Kg bee package, a normal feedstuff consumption rate is about 110 g/day. If ambient temperature is below 4°C, bees cannot control their box temperature, loosing heat and eventually die. e) Feedstuff shortage can produce first an increase in temperature, followed by a fast drop of it until bees' death, simultaneously loosing relative humidity control. d) A normal CO<sub>2</sub> concentration inside the box ranges between 450 to 1500 ppm, however, containers can be limited in their CO<sub>2</sub> control capacity if the cargo is more than 500 bee packages. e) Once back in the field, Bees' colonies success is associated to the colony size. We thank the FIA Grant PYT-2020-0283.





### PP-419

#### Physical and chemical characterization of royal jellies produced in different regions of Chile and their comparison with royal jellies purchased in the informal trade

Karen Delpilar Yañez Arellano<sup>1</sup>, David González<sup>2</sup>, Daniel Ramírez<sup>2</sup>, María Inés Carvallo<sup>3</sup>, Pamela Valdés<sup>4</sup>

<sup>1</sup>Centro de Biotecnología Dr. Daniel Alcalay Lowitt, Universidad Técnica Federico Santa María, Valparaíso, Chile

<sup>2</sup>Departamento de Ingeniería Química y Medio Ambiente, Universidad Técnica Federico Santa María, Valparaíso, Chile

<sup>3</sup>Agrícola y comercializadora Apipucalán Limitada, Nogales, Chile

<sup>4</sup>Panales del Sur Limitada, Rancagua, Chile.

In the current context of climate change, where honey production is decreasing, national beekeeping needs to diversify its products, of which the production of royal jelly is a good alternative, little exploited by national beekeepers and in great demand, since it is a complete food supplement and active ingredient used in dermocosmetics and pharmacology. Despite its high demand, there is no information about the physical and chemical composition and quality of royal jellies produced in Chile, so this study consisted of physically and chemically characterizing the composition of three royal jellies produced in different regions of the country in hives fed naturally, one lyophilised royal jelly and one informal trade royal jelly to know the variability in the content of total phenols, flavonoids, B vitamins and 10- hydroxydecenoic acid (as an international measure of quality of royal jellies). The objective of this study was to establish differentiation parameters of royal jellies produced in different regions of Chile and to compare them with a sample of freeze-dried royal jelly and a royal jelly obtained in the informal trade.

### PP-420

#### Antioxidant powdered beverage based on honey and native plants from Argentina (*Zuccagnia punctata* and *Solanum betaceum*) obtained by spray drying

Florencia María Correa Uriburu<sup>1</sup>, Iris Catiana Zampini<sup>3</sup>, Luis Maldonado<sup>2</sup>, Maria Ines Isla<sup>3</sup>

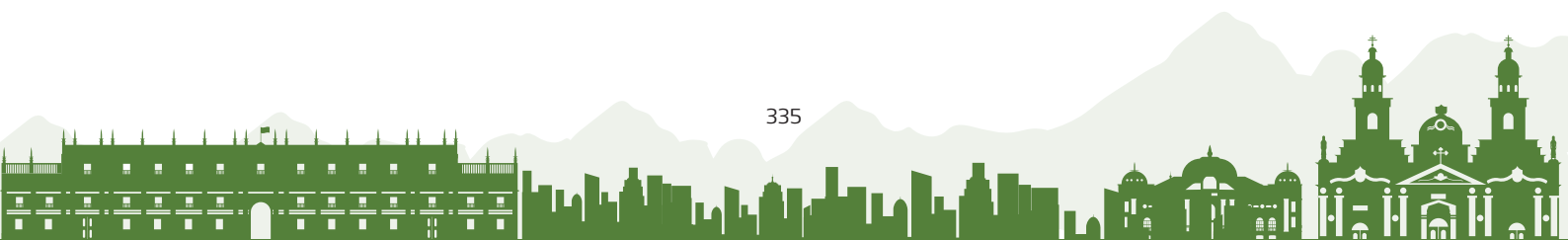
<sup>1</sup>Instituto de Bioprospección y Fisiología Vegetal (INBIOFIV), CONICET-Universidad Nacional de Tucumán (UNT), San Miguel de Tucumán T4000CBG, Argentina, Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria, Famaillá, PROAPI, Ruta Provincial 301-km 32, Famaillá 4132, Tucumán, Argentina

<sup>2</sup>Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria, Famaillá, PROAPI, Ruta Provincial 301-km 32, Famaillá 4132, Tucumán, Argentina

<sup>3</sup>Instituto de Bioprospección y Fisiología Vegetal (INBIOFIV), CONICET-Universidad Nacional de Tucumán (UNT), San Miguel de Tucumán T4000CBG, Argentina, Facultad de Ciencias Naturales e Instituto Miguel Lillo (UNT). S. M. de Tuc. T4000 JFE. Argentina

Functional beverages contain specific ingredients that are added to provide an additional health benefit, such as vitamins, amino acids and phenolic compounds. The main sources of phenols in functional beverages are fruits, vegetables and native medicinal plants. *Zuccagnia punctata* (jarilla, pus pus, lata) and *Solanum betaceum* (chilto, tree tomato) contain large amounts of antioxidant compounds, mainly chalcones, anthocyanins and rosmarinic acid derivatives. On the other hand, honey is rich in vitamins and minerals, such as vitamin B, calcium and iron, which can provide important nutrients to the body. It has antioxidant activity that helps protect the body against cell damage and reduce inflammation, as well as providing a natural source of carbohydrates, which provide energy to the body quickly and effectively.

The present study used Citrus limon monofloral honey as a natural sweetener, *Z. punctata* extract and chilto juice for the preparation of an antioxidant beverage. Each ingredient (*Zp* extract, chilto juice and honey) was obtained according to the Argentine Food Code and characterized. The beverages were formulated using maltodextrin (MD) with two dextrose equivalents (DE), 10 and 15, and then spray-dried at an inlet air temperature of 130 °C. The physicochemical, microscopic, phytochemical and functional characteristics of the powders were determined. The results showed good physical properties for both formulations showing high water solubility with characteristics suitable for handling, transport and storage. The chromatic parameters of both powdered beverages indicate orange-pink tones regardless of the wall material used. The total content of polyphenols and flavonoids in the beverages was maintained after spray drying. Anthocyanins were less stable under drying conditions. Both powdered beverages showed high scavenging capacity of ABTS•+, HO• and H<sub>2</sub>O<sub>2</sub> (SC<sub>50</sub> between 3.29 and 41.05 µg GAE/mL) and were able to inhibit xanthine oxidase (XOD) activity (CI<sub>50</sub> between 91.35 and 114.43 µg GAE/mL). The beverages were neither toxic nor mutagenic in the biologically active concentration range. The results obtained in the present work support scientifically the use of the powdered beverages of Argentinean native plants as antioxidant and Citrus limon monofloral honey as natural sweetener.





### PP-421

### Study of the Antimicrobial and Antibiofilm Potential of Honey from four Different Stingless Bees from Yucatan Peninsula and their Differentiation through Electrophoretic Profiles

Elizabeth Ortiz-Vázquez<sup>1</sup>, Lenny Suarez Caamal<sup>1</sup>, Jesus Manuel Ramon Sierra<sup>1</sup>, Erick Cuevas Moguel<sup>1</sup>, Alejandro Yam Puc<sup>1</sup>, Aurora Xolalpa Aroche<sup>2</sup>

<sup>1</sup>División de Estudios de Posgrado e Investigación, Tecnológico Nacional de México/ITMérida, Mérida, México

<sup>2</sup>Universidad Intercultural Maya de Quintana Roo, José María Morelos, México.

Honey from stingless bees is highly valued for its medicinal properties, especially that of the bee *Melipona beecheii*, which is used as a treatment for bacterial infections of the respiratory tract, gastrointestinal tract, and dermatological problems. Recent studies have shown that this honey owes its biological properties to compounds of the phenolic and protein type. It is important to mention that in the Yucatan peninsula there are other honeys from stingless bees. About 18 species of stingless bees have been reported, which are attributed several medicinal properties. Therefore, the objective of this work was to evaluate the antimicrobial and antibiofilm activities of four honeys from stingless bees *Scaptotrigona pectoralis*, *Cephalotrigona zexmenie*, *Nannotrigona perilampoides* and *Frieseomelitta nigra* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and to determine honey authenticity using their protein profile.

Protein extract (PE) of the honeys from four stingless bees was obtained by ultrafiltration, then they were analyzed using an SDS-PAGE electrophoretic system. These analyses showed that the protein profiles of the honeys were different; although some protein bands between 4 and 8 KDa were similar. All the honeys inhibited the growth of both bacteria *S. aureus* and *P. aeruginosa*, at concentrations below 30%. When evaluating the minimum inhibitory concentration of honeys, *C. zexmenie* honey had the highest activity against *S. aureus*, while the *N. perilampoides* honey for *P. aeruginosa* both of them required 5% of honey to inhibit 100% bacterial growth. Every PE of the honeys inhibited the growth of both evaluated pathogens. *F. nigra* honey PE inhibited 100% of the growth of *S. aureus* with a MIC of 7.2 µg/mL; while *C. zexmenie* honey PE inhibited the growth of *P. aeruginosa*, which was more susceptible requiring a MIC of 3.6 µg/mL. In the case of antibiofilm activity, all honeys inhibited the formation of the biofilm around 1% concentration, *S. pectoralis* and *N. perilampoides* honeys presented higher antibiofilm activity. The stingless bee honeys evaluated in this study have a great potential to be used as antimicrobial agents and they could be authenticated by electrophoretic analysis.

### PP-422

### Evaluation of preservation methods for products derived from native stingless bee colonies

Juan Carlos Mayo Velazquez<sup>1</sup>, José Fausto Rivero Cruz<sup>2</sup>, Alejandro Antonio Aragón Moreno<sup>3</sup>, Lizbeth Areli Chimal Cahuich<sup>1</sup>, Edward Emmanuel Brito Estrella<sup>1</sup>, Jesús Alejandro Yam Puc<sup>4</sup>, Aurora Xolalpa Aroche<sup>1</sup>

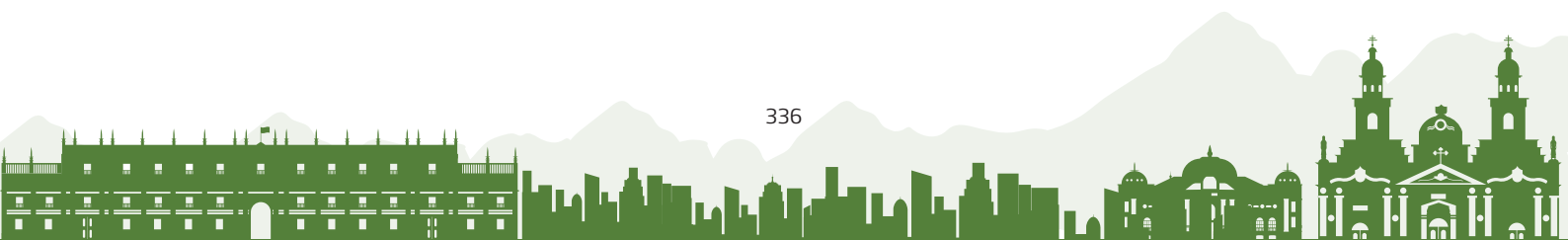
<sup>1</sup>Centro de Innovación para el Desarrollo Apícola Sustentable, Universidad Intercultural Maya de Quintana Roo, La Presumida, José María Morelos, Quintana Roo, México.

<sup>2</sup>Finca la Isla SPR de RL, Papantla, Veracruz, México.

<sup>3</sup>El Colegio de la Frontera Sur, Unidad Chetumal, Chetumal, Quintana Roo, México.

<sup>4</sup>Instituto Tecnológico de Mérida, Mérida, Yucatán, México.

Out of the more than 20,000 species of bees existing in the world, at least 1,800 are recorded in Mexico, which 90 is from Quintana Roo, that is closely related to the meliponiculture or breeding of the stingless bee *Melipona beecheii* and other species belonging to the Meliponini tribe, due to its importance for traditional family life and contributing to the conservation of native bees, with records dating back to pre-Hispanic times. The objective of this work focuses on the evaluation of methods to preserve products derived from the colony, a prior to that, the characterization of honey and geopropolis was taken from 7 meliponaries established in semi-urban and rural areas in the municipalities of Felipe Carrillo Puerto, Q. Roo and José María Morelos, Q. Roo, with low disturbance levels. Initially, physicochemical parameters in the honeys were evaluated, such as humidity levels with a manual refractometer, pH and conductivity levels using a potentiometer, as well as color with a colorimeter that yields results in degrees Pfund and the scale included in the equipment is used to set the color. The influence of floral resources was determined through a melisopalynological analysis using the acetolysis method for both honey and geopropolis, lastly requiring a pretreatment to eliminate residues that could react with acids, for this, a previous washing with 10% KOH was used. As a result, most of the evaluated honeys have humidity ranging between 23% and 25%, with light tones and at least four botanical families were identified, mainly Asteraceae, Myrtaceae and Burseraceae, in particular the case of geopropolis, it was required to multiply the samples mounted because of the low percentage of pollen that composes it. Finally, this information is returned to the communities as part of the effort for the conservation and revaluation of meliponiculture in the Mayan zone of the state.



### PP-423

#### Botanical and physicochemical typing in honey samples produced in the Biobío Region, Chile

Jorge Mauricio González, María Paz Molina, Hernán Eduardo Soto, Laura Milena Koch, Tamara Monserrat Vera  
Línea de Conservación y Mejoramiento Genético, Instituto Forestal

As part of the Honey Flora Program executed by the Forest Institute (INFOR), during the period spanning from 2016 to 2022, a total of 199 honey samples sourced from beekeepers in the Biobío Region (Chile) have been meticulously collected. These samples underwent meticulous botanical classification through the utilization of melissopalynology methodologies. Furthermore, they underwent a rigorous typification process based on critical physicochemical parameters deemed essential for their commercial viability, conforming to both national and international regulations.

The assessed parameters were subjected to thorough multivariate analysis in order to furnish supplementary insights concerning the quality and comprehensive characterization of the honey produced within the region. Scrutiny of adherence to national and international regulations is conducted, and notable strides in the implementation of spectroscopy techniques and chemometric methodologies, positioned as alternative means of honey characterization, are presented.

A careful examination of the botanical classification of honey was undertaken to glean insights into the territorial diversity of melliferous species prevalent within the region.

The findings derived from these analyses are actively being leveraged to enhance the operational efficacy of the Program. To date, these findings have yielded a profound understanding of the quality parameters associated with honey production in the region, while concurrently identifying gaps warranting remedial action in meeting prescribed quality standards. Moreover, regions necessitating supplementation of melliferous flora have been ascertained, alongside the identification of monofloral honeys, particularly emphasizing the inherent value intrinsic to native monofloral honey varieties.

### PP-424

#### Volatile Constituents and Biological Activities of Brown Mexican Propolis

José F Rivero<sup>1</sup>, Mariana Guerra Montiel<sup>2</sup>, Blanca E Rivero Cruz<sup>2</sup>, Adriana Correa Benítez<sup>3</sup>, Aurora Xolalpa Aroche<sup>4</sup>

<sup>1</sup>Finca la Isla SPR de RL, Papantla, Veracruz, México

<sup>2</sup>Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Ciudad de México, México

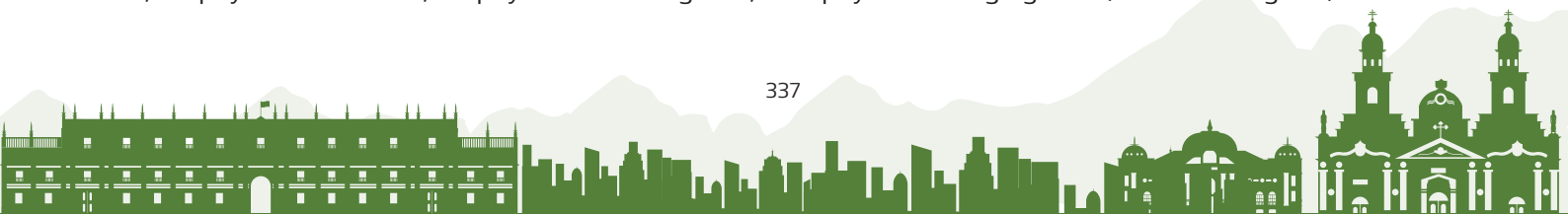
<sup>3</sup>Facultad de Medicina Veterinaria y Zootecnia, Ciudad Universitaria, Ciudad de México, México

<sup>4</sup>Ingeniería en Sistemas de Producción Agroecológicos. Universidad Intercultural Maya de Quintana Roo (UIMQROO). Carretera Muna-Felipe Carrillo Puerto Km 137, La Presumida, José María Morelos, Quintana Roo, México

*Apis mellifera* L. propolis, known as the bee glue, combines resins collected by the honeybees from different plant organs, and with beeswax that honeybees additionally incorporate. It is a resinous mixture containing polyphenols, mainly flavonoids and phenolic acids as well as volatile compounds. It has been shown that propolis possesses antioxidant, antibacterial, antifungal, and antiviral properties, as well as other beneficial biological activities such as anticancer, immunostimulant, anti-inflammatory, antiulcer, hepatoprotective, antidiabetic, etc.

Since the 1960s, numerous studies have revealed propolis composition variability with more than 300 natural organic compounds: flavonoids, phenolic acids and their esters, polyphenols, terpenes, lignans, steroids, hydrocarbons, amino acids, and others. Their abundance has been influenced by botanical and geographical factors, as well as by the season. According to the chemical composition, different types of propolis are described: (a) Poplar type (*Populus* spp.), (b) Birch type (*Betula verrucosa* Ehrh.), (c) Green type (*Baccharis* spp.), (d) Red type (*Dalbergiaspp.*), (e) Clusia type (from *Clusia* spp.), (f) Pacific type (*Macaranga tanarius*(L.) Mull.Arg.) and (g) Mediterranean type (mostly from *Cupressaceae*/*Juniperus* family). Propolis of the European poplar and Brazilian red and/or green (*Baccharis* spp.) types have been widely studied; on the other hand, the chemical composition of propolis collected in Mexico is scarce. Twelve different propolis samples collected from North and Mexican Altiplano regions were chemically and biologically evaluated. Volatile compounds of the samples were analyzed by headspace-solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). The main volatile constituents were identified as  $\alpha$ -pinene (33.04%),  $\beta$ -pinene (20.18%),  $\alpha$ -phellandrene (20.2%), nonanal (18.83%), octanal (14.5%),  $\gamma$ -terpinene (13.57%), 1,8-cineole (12.53%),  $\delta$ -3-carene (12.53%),  $\gamma$ -cadinene (9.54%),  $\alpha$ -terpineol (6.75 %), (2E)-heptenal (5.4%), for the propolis samples.

The antimicrobial activity was determined using the microdilution method in a 96-well plate and *Staphylococcus aureus*, *Staphylococcus oralis*, *Staphylococcus sanguinis*, *Phorphyromonas gingivalis* (0.01 to 1.2 mg/mL).



### PP-425

#### Evaluation of Quality Parameters of Stingless Honey produced in Veracruz and Quintana Roo, Mexico

Blanca E Rivero Cruz<sup>1</sup>, José F Rivero<sup>2</sup>, Emil Neme Martínez<sup>2</sup>, Aurora Xolalpa Aroche<sup>3</sup>

<sup>1</sup>Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Ciudad de México, México

<sup>2</sup>Finca la Isla SPR de RL, Papantla, Veracruz, México

<sup>3</sup>Ingeniería en Sistemas de Producción Agroecológicos. Universidad Intercultural Maya de Quintana Roo (UIMQROO). Carretera Muna-Felipe Carrillo Puerto Km 137, La Presumida, José María Morelos, Quintana Roo, México

Stingless bee honey is a product obtained from plant nectar and exudate or excretion of plant-sucking insects collected by bees belonging to the tribe Meliponini, subfamily Apinae. As the popular name suggests, stingless bees are so-called as they have vestigial (atrophied) sting, unlike the *Apis mellifera* bees.

Stingless bee honeys have higher moisture content and acidity and lower sugar content, diastase activity, and 5-hydroxymethylfurfural (5-HMF). These parameters do not fit the limits imposed by the current regulation in Mexico. This product is usually sold informally, directly by producers, without any certificate of quality and authenticity, being subject to fraud. Due to its uniqueness, stingless bee honeys are attracting consumers who are looking for a less sweet, more acidic product with a unique aroma and a lighter colour.

Stingless honeys produced by *Scaptotrigona mexicana* and *Melipona beecheii* contain active compounds with pharmacological activities. These characteristics reflect the rich composition of phenolic acids, flavonoids, and volatile compounds that they possess. The composition of stingless honey varies depending on the geographical area from which they are obtained. Therefore, the main physicochemical characteristics of 100 honey samples (color, acidity, pH, total sugars, conductivity, diastase index, HMF content and humidity), their antioxidant activities (total phenolic content, total flavonoid content, and DPPH assay) and antibacterial characteristics against (*Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus sanguinis*, *Porphyromonas gingivalis*) were determined. All the honeys complied with the standards established in the literature for stingless honey.

### PP-426

#### Pollen spectra of honeys from main harvest produced at the protected area of Montes del Queguay, Uruguay

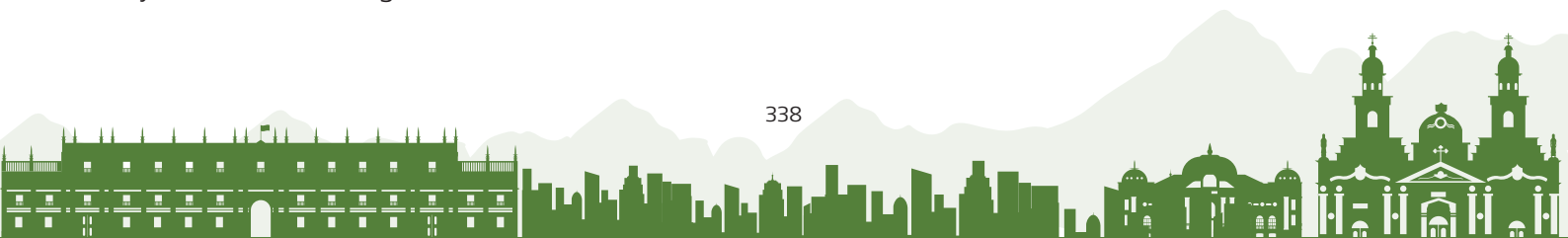
Gloria Daners<sup>1</sup>, Selva Cora<sup>2</sup>, Gabriela Tamaño<sup>2</sup>, Eduardo Dellacassa<sup>3</sup>

<sup>1</sup>Instituto de Ciencias Geológicas, Facultad de Ciencias, UdelaR, Iguá 4225, 11400-Montevideo, Uruguay

<sup>2</sup>Laboratorio de Tecnología de la Miel y Productos Apícolas - ApiUTEC - LAA-ITRSO-UTEC (Universidad Tecnológica), Av. Salto 919, 60000-Paysandú, Uruguay

<sup>3</sup>Laboratorio de Biotecnología de Aromas-DQO-CYTAL, Facultad de Química-UdelaR, Gral. Flores 2124, 11800-Montevideo, Uruguay

The pollen content of 13 honey samples from beehives located in the Protected Area of Montes del Queguay, Paysandú Department in Uruguay, was analyzed. Being originated from a protected area system (SNAP), the country authorities control the land use and the sustainability of areas of specific interest according to defined characteristics. In particular, Montes del Queguay, area included in this system in 2014, stands out for containing a great diversity of environments, types of vegetation and the presence of priority species for conservation. Likewise, it includes one of the largest masses of natural riverside forests in the country. These factors determine of interest the analysis of the products obtained within it as a way of seeking the sustainability of the ecosystems and valorization of, in this case, its honeys. The samples here studied were harvested in February-March of 2022, corresponding to the main harvesting period in this region. For the concentration of pollen, standard melissopalynological procedures with acetolysis were used. A total of 84 pollen types were identified (32 at species level, 25 at genus, 21 at tribe or family, and 6 remain undetermined). The palynological spectra showed dominance of pollen from native plants whose accumulated frequency varied between 47 and 93%. In more than 60% of the samples, the native trees *Scutia buxifolia*, *Blepharocalyx salicifolius* and *Terminalia australis* represented more than 74% of the spectrum. The group made up of the native tree species *Scutia buxifolia*, *Blepharocalyx salicifolius*, *Terminalia australis*, *t. Schinus sp.*, *Salix sp.*, along with the prairie species *Lotus sp.*, were found to be present in 100% of the samples. In turn, the presence of monofloral honeys from *Scutia buxifolia* (2 samples) and *Blepharocalyx salicifolius* (2 samples) stands out. Based on the data obtained, it can be assumed that the honey samples characterization from the pollen point of view in the Montes del Queguay Protected Area would be feasible. In order to obtain statistically representative information, it will be necessary to analyze honeys harvested in other years at the same season, as well as those harvested in different times of the year at the same region.



## Beekeeping Technology and Quality

PP-427

### The Biofactory of Native Bees: rescuing, keeping and multiplying wild stingless bees to preserve biodiversity for future income generation in communities in southeast Amazon

Juliana Stephanie Galaschi-Teixeira<sup>1</sup>, Jamille Veiga<sup>1</sup>, Luciano Costa<sup>2</sup>, Vera Lucia Imperatriz Fonseca<sup>3</sup>, Valeria Tavares<sup>1</sup>

<sup>1</sup>ITV Sustainable Development, Belém, Brazil

<sup>2</sup>Universidade Federal do Pará, Belém, Brazil

<sup>3</sup>Universidade de São Paulo, São Paulo, Brazil

Stingless bees are a diverse group of social insects with great ecological and socioeconomic importance for pollination of wild and cultivated plants, and production worldwide consumed bee products, like honey and propolis. Native colonies have been rescued from areas of vegetation suppression related to mining operations at Serra dos Carajás (in the Amazon Forest biome southeast of Pará, Brazil) but without a clear plan of what to do next. Since the problem concerned the underuse of such valuable native resources, the Biofactory of Native Bees was born as a natural solution to rescued colonies. At the Biofactory, we keep wild colonies and multiply them using traditional and new breeding techniques, applying a research-driven approach to colony management. The project started in 2019 in Serra dos Carajás and is ongoing. During the past four years, we rescued 70 colonies of 22 species of stingless bees. The most abundant species were the Uruçu-boca-de-renda (*Melipona seminigra*) and the Iraí bee (*Nannotrigona punctata*), essential for honey production and crop pollination in the region. We also established three meliponaries: with a starting point of two species and 20 colonies, meliponaries now comprise five species (*Melipona seminigra*, *Nannotrigona punctata*, *Plebeia alvarengai*, *Scaptotrigona postica* and *Tetragonisca angustula*) and a total of 80 colonies kept in hive boxes. The Biofactory of Native Bees impacts the Carajás region by preserving the genetic diversity of wild populations and aims to expand that by generating income for local communities. By making the produced colonies available for breeding in the future, we expect to connect biodiversity, conservation and society in the Carajás region, and serve as a model to inspire other socioeconomic initiatives in the Amazon.

PP-428

### 'Optimisation of bee venom collection technology'

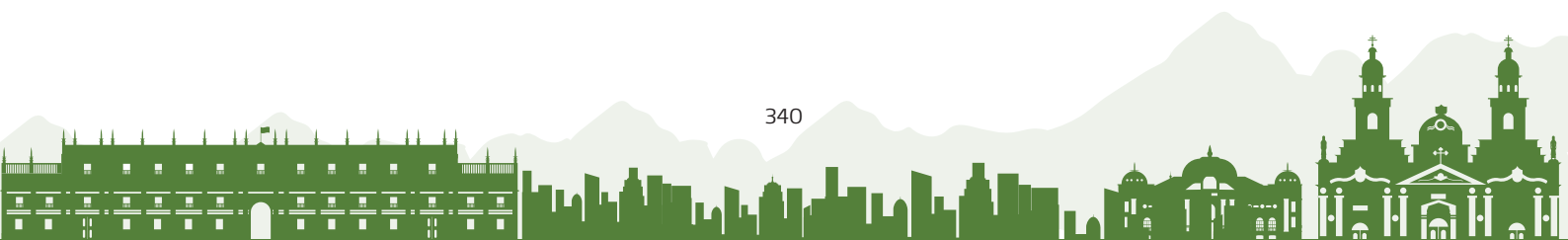
Simon Golob

Slovenian Beekeepers' Association

Bee venom is a secretion produced by the acid gland of honeybees. Honeybees use their venom to defend the hive. Just like other bee products, bee venom is used by humans for a variety of purposes. In order to make the production of bee venom more accessible, easier and ultimately more optimal for beekeepers, the Slovenian Beekeepers' Association has launched a three-year research project in 2020 entitled 'Optimisation of bee venom collection technology'. As part of this research, bee venom was extracted from honeybee colonies in standard Slovenian AŽ hives and in standard LR hives, which were located in the same location in the central Slovenian region.

In the research, we compared different parameters and their impact on the quantity and quality of the venom and on the behaviour of the bees during the operation of the bee venom collectors. We compared standard Slovenian AŽ Hives and standard LR hives, venom extraction at the hive entrance of AŽ and LR hives, at the place of combs in both hive systems, behind lower brood chamber in the Slovenian AŽ hives and above the honey super in the LR hives. We compared different duration of venom extraction (30 and 45 minutes) and the influence of colonies and their characteristics on venom extraction. Strength, calmness, brood quality, honey and pollen supply in combs was monitored throughout the study.

The results showed, that venom extraction is better in LR hives, that from the point of view of aggression of bees at the time of extraction, and from the point of view of the quality and quantity of venom, it is better to place bee venom collectors inside of the hives - above the honey super in the LR hives and behind lower brood chamber in AŽ hives. We found that venom extraction does not have a negative impact on the monitored colonies characteristics and that there are large differences in the amount of venom extracted between some colonies.





### PP-429

#### Quality of royal jelly produced by Africanized *Apis mellifera* in southern Brazil

Alessandra Fernandes Gonçalves Benites<sup>1</sup>, Douglas Galhardo<sup>1</sup>, Cibele Regina Schneider<sup>1</sup>, Daiani Rodrigues Moreira<sup>2</sup>, Adriana A. Sinópolis Gigliolli<sup>2</sup>, Vagner de Alencar Arnaut de Toledo<sup>3</sup>

<sup>1</sup>Postgraduate Program in Animal Science, State University of Maringá, Maringá, Brazil

<sup>2</sup>Department of Biotechnology, Genetics and Cell Biology, State University of Maringá, Maringá, Brazil

<sup>3</sup>Professor at the State University of Maringá, Animal Science Department and Postgraduate Program in Animal Science

The queen is of great importance because she gives rise to all individuals in the colony. Thus, the quality of the queen directly interferes with the development of the colony, health and productivity. Therefore, the production of quality queens is of great interest to beekeepers. In this study, it was tested that the volume of the cup interferes with the quality of the queen, due to the hypothesis that the amount of royal jelly deposited in the different volumes of cups would influence the nutrition of the larvae, interfering with the quality of the queens produced. For this, the production of queens was carried out using five cups volumes (602  $\mu$ L, 702  $\mu$ L, 802  $\mu$ L, 901  $\mu$ L, 1002  $\mu$ L). For the production of queens, three production cycles were performed, using 15 colonies, with three colonies for each cup volume. Each colony had a cup frame with three bars, with 14 cup each, totaling 42 cups per colony. After emergence, the queens were identified in the upper part of the thorax and placed in queen cages containing candy and companions, being stored for one to three days in a B.O.D. on controlled environmental conditions for further morphological analysis of the ovaries. The queens were dissected, and the ovaries were prepared for Light microscopy and Scanning Electron Microscopy of the ovaries of virgin queens. The production of queens evaluating the different volumes of cups did not interfere with the morphology of the ovaries of Africanized *A. mellifera* queens. The data from this study are of great importance for commercial beekeeping that seeks better queens for its colonies and the commercialization of quality queens.

### PP-430

#### Physical, chemical and palynological characteristics of honeys produced in the south of the province of Buenos Aires, Argentina

María Soledad García Paoloni<sup>1</sup>, Graciela A. Rodríguez<sup>1</sup>, Mónica Gaggiotti<sup>2</sup>, Laura Barrionuevo<sup>3</sup>, Victor Alberto Rusas<sup>4</sup>

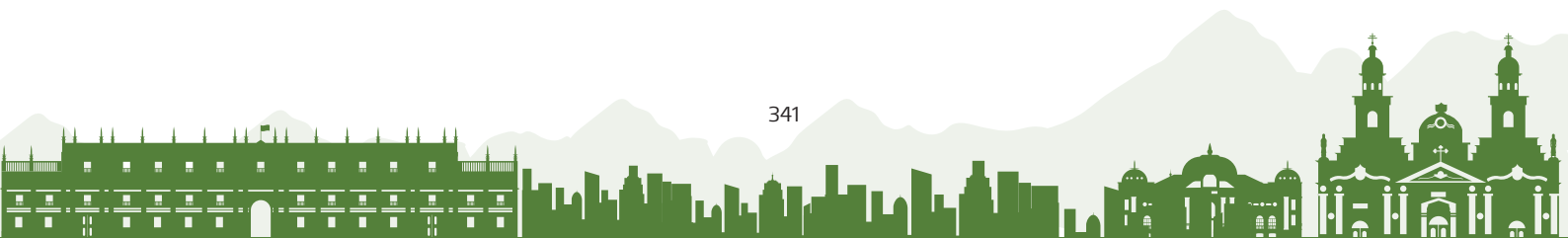
<sup>1</sup>Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Hilario Ascasubi, Argentina

<sup>2</sup>IDICAL-CONICET, INTA, EEA Rafaela, Argentina

<sup>3</sup>Laboratorio de Melisopalinología, Bahía Blanca, Argentina

<sup>4</sup>Cambio Rural – INTA, EEA Colonia Benítez, Argentina

The south of the province of Bs As (SPBA) belongs to the phytogeographic regions of Monte and Espinal in accordance to Cabrera, although the vegetation constitutes an ecotone between both. It has a semi-arid climate with an average annual temperature of 15°C and annual rainfall between 100 and 400 mm. The main economic activity is agricultural and livestock production, especially in the valleys irrigated by the Colorado and Negro rivers. An important beekeeping activity has also been carried out since the middle of the last century. With the objective of evaluate the physical-chemical and palynological characteristics of the honeys produced in this territory 18 samples were analyzed, supplied by beekeepers during the productive period of 2020/21. The studied parameters were colour, moisture, hydroxymethylfurfural (HMF) and electrical conductivity (IRAM methodology), also glucose/fructose ratio (UV method). Regarding the first, the values ranged between 17.0 and 67.7 mm Pfund, but 72% of the samples were classified as "white honey" according to the international color scale. All moisture values obtained were below 20% in accordance with the Mercosur technical regulation, and the conductivity showed lower values than 0.4 mS/cm wich corresponds to "flower honey" according to the Codex Alimentarius. In general, the HMF content was less than 29 mg/kg; only one sample exceeded the standard limit value with 85 mg/kg. Related to melisopalinological analyses, 39% of the samples can be classified as multifloral and the remaining 61% as monofloral (55% Brassicaceae - 6% Neltuma sp.). At the end of the season, *Diplotaxis tenuifolia* (Dt) dominates the area over other flowering, reason why monofloral honey of this taxon can be obtained if temperature and ambient humidity conditions are optimal. It shows a very light color (16.0 to 18.0 in this study) and fast crystallization (most samples presented F/G values less than 1.1). Based on the obtained results, monofloral Dt honey could be obtained in this territory, although it would be necessary study at least another two seasons to evaluate the effect of meteorological variations. Finally, physical, chemical, functional and sensory characterization of Dt honey could add value to beekeeping production in SPBA region.





### PP-431 Solar Energy Apicultural Drinking Fountain

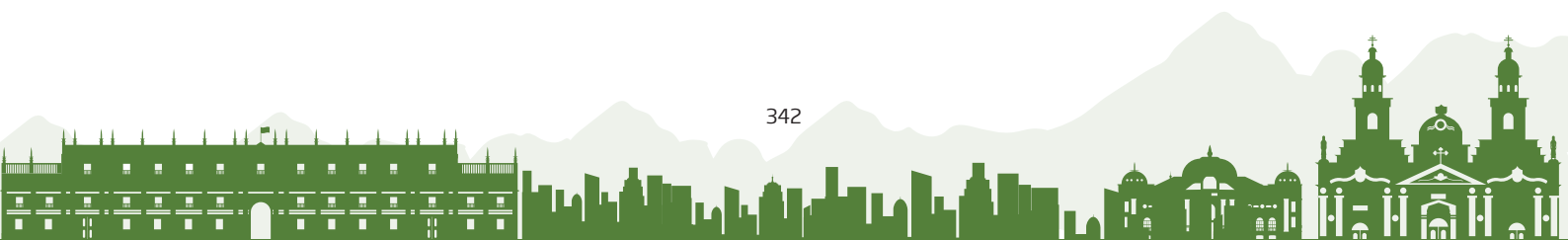
Osvaldino Rosa Filho<sup>1</sup>, Lucas Boeira Michels<sup>2</sup>, Miguelangelo Ziegler Arboitte<sup>3</sup>

<sup>1</sup>Instituto Federal de Santa Catarina Campus Araranguá

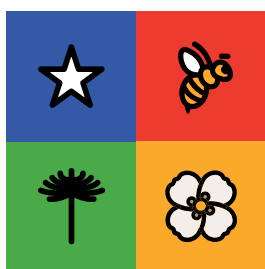
<sup>2</sup>Autônomo

<sup>3</sup>Instituto Federal Catarinense Campus Santa Rosa do Sul

In semi-arid climates, low air humidity, high temperatures and lack of water cause mortality of immature offspring and the abandonment of swarms and bee nests. Food and water supply ensure the survival and population growth of *Apis mellifera* colonies. Researchers from the Federal Institute of Science and Technology of Santa Catarina – Araranguá, and the Federal Institute of Santa Catarina Campus Santa Rosa do Sul – Brazil, evaluate the thermodynamic cycle of the collective water trough to solar energy and the behavior of collecting bees. The experimental prototypes derive from a black cylinder that absorbs thermal energy, raising the liquid under pressure to collective diffusion distributors or closed circulation. The model is self-supplied with rain, pouring water in the hottest hours of the day, recovering surpluses with maximum economy and autonomy for long periods. The functioning of the drinking fountain responds to the regularity of the solar transit and local meteorological variations, describing flow curves similar to the nectar secretion flows and in close synchrony with the flight frequency of the bees, offering water in exponentially increasing volumes, until exhaustion, replenishment or natural replenishment of content. The long duration of the cycle accompanies the seasonal growth of the colonies and centralizes the source in the apiary, reducing the wear of the bees in the thermal regulation of the hives and conservation of the humidity of the nest. The flow of the drinker is determined by the thermal amplitude, radiation, initial volume, pressure and density of the working fluids, and can be adjusted by the position and geographic location, observing the climatological normals, flowering calendars, forecasts of water deficit or eventual emergencies. The simplicity of the system and the hygienic-sanitary control of the process recommend its experimental use a priori. The method under development will be able to provide stability and balance to apiaries in critical situations, without opening the hives - non-invasive - justifying the continuity of studies of the resources and possibilities of the invention. Equating the hypothesis that even unstable atmospheric conditions can be sufficient for the external activity of the bees and the regular and shared supply of water or food - without significant losses or damages - constitutes the main objective of the project.







# APIMONDIA

48<sup>TH</sup> INTERNATIONAL **CHILE**  
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September 4<sup>th</sup> - 8<sup>th</sup>, 2023

*Sustainable Beekeeping, from the south of the world*

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