Third FAO/IAEA International Conference on

Area-Wide Management of Insect Pests:

Integrating the Sterile Insect and Related Nuclear and Other Techniques

BOOK OF ABSTRACTS

Organized by the

FAO FIAT PANIS

IAEA

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

22–26 May 2017
Vienna, Austria

CN-248

Organized by the

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Table of Contents

Session 1: Operational Area-wide Programme

Abstract ID 174

Past, Present and Future: A Road Map to Integrated Area-wide Systems and Enterprise Risk Management Approaches to Pest Control

Kenneth BLOEM

Science and Technology, USDA, APHIS, PPQ, Raleigh, North Carolina, USA, Email: *kenneth.bloem@aphis.usda.gov*

Integrated pest management (IPM) is the careful consideration of all available pest control tactics and the subsequent integration of appropriate measures in a coordinated fashion to manage pests below economic levels, wherein individual producers practice independent pest control over localized populations.

Area-wide (AW) integrated pest management programmes on the other hand tend to focus on a single key pest and integrate multiple tactics applied to the total pest population in all of an ecosystem. More recently, systems approaches to pest management attempt to apply mitigation measures at all steps along the pathway from field to table, including pre- and post-harvest treatment options.

This talk will provide an overview of each of these topics and their interrelationships in the context of international trade and a global environment, including regulatory and legal issues. It will discuss the need to inform the public and coordinate stakeholders using and integrating many different methods or tactics such as host plant resistance, selective insecticides, mating disruption, biological control and SIT. It will discuss the concepts of non-host status, areas of low pest prevalence, preventive control using early warning systems and off-shore risk mitigation, and the use or creation of refugia to manage resistance or maintain biological control agents. Examples will be given involving field crops, orchards, forests, the livestock sector and human disease vectors.

Finally, this presentation will explore ideas and opportunities to leverage the significant infrastructure needed to deliver an AW programme to broaden the services they provide and thereby the stakeholder groups who can undertake these programmes.

Key words: Area-wide Programmes, Integrated Pest Management, Systems Approach, Risk Management

Abstract ID 246

Technological Innovations in Global Desert Locust Early Warning

Keith CRESSMAN

FAO, Rome, Italy, Email: *keith.cressman@fao.org*

The Desert Locust *Schistocerca gregaria* Forsskål is considered to be the most dangerous of all transboundary migratory insect pests. It can affect up to 20 percent of the Earth's land surface and threaten crop production and food security on a continental scale.

One of the core mandates of the Food and Agriculture Organization of the United Nations (FAO) is to monitor Desert Locusts throughout the world and provide early warning to affected countries and the international donor community. This task has been delegated to the *Desert Locust Information Service*, which operates an early warning system that consists of dozens of networked countries and latest technologies and innovations in communications, remote sensing, data management and spatial analysis.

Early warning is a key requirement in preventive control to reduce the frequency, intensity and scale of devastating locust plagues. More than 50 years of lessons learnt from FAO's successful Desert Locust early warning system can be applied to other pests and diseases.

Abstract ID 271

Area-wide Management of Rice Insect Pests in Asia through Integrating Ecological Engineering Techniques

Kong Luen HEONG

Centre for Agricultural BioSciences International (CABI), South East Asia Regional Centre, P. O. Box 210, 43400 University Putra Malaysia (UPM), Serdang, Malaysia, Zhejiang University, Hangzhou, China, Email: *klheong@yahoo.com*

Most rice insect pests are exogenous immigrants from either long distances or neighbouring fields. For their management to be economical and sustainable, an area-wide prospective is imperative. Key pests, like the planthoppers and stem borers are monophagous and are dependent on rice for survival and reproduction. They multiply and move from one rice crop to another, sometimes carrying virus diseases such as ragged stunt, grassy stunt and rice stripe from source areas. The planthoppers are *r* strategists, unable to overwinter and are known to "migrate" or are displaced by wind from Southern China to temperate regions of China, Japan and Korea.

With adequate faunal biodiversity and biological control ecosystem services in a rice crop, immigrant pests have low chances of survival and growth capacities and often remain as minor pests. However, when the local ecosystem services are compromised, often by unnecessary insecticide use or extreme weather conditions such as droughts or floods, the immigrants would have high survival and growth rates. From 2008 the Rice Bowl of Thailand suffered brown planthopper (BPH) *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) outbreaks for 14 consecutive seasons that caused losses of more than US$ 200 million. Farmers were routinely applying insecticides as prophylactics and the BPH "escaped" its natural control and populations increased 100,000 fold.

Ecological engineering (EE) approaches involve practices that would increase biodiversity and ecosystem services and reduce insecticide threats to ecosystem services. An area-wide increase in floral biodiversity in the crop landscape would provide Shelter, Nectar, Alternate hosts and Pollen (abbreviated as SNAP) to conserve the natural enemy fauna that provides the natural control ecosystem services. Pioneered in Jin Hua, China with sesame plants grown on the paddy bunds or dikes, EE is now practiced in Thailand, Vietnam, Philippines and China using several flower species.

A multi-country, multi-year field trial conducted by IRRI scientists in collaboration with scientists from Australia, China, Thailand and Vietnam showed that the growing of flowers on rice bunds as an EE practice increased profits (by 7.5%), increased yields (by 5%), increased biological control (by 45%) and added aesthetic values to the rural landscape. At the same time the EE practice decreased insecticide use (by 70%), decreased pest densities (by 30%) and farmers' chemical input costs (70%).

Farmers are adopters and implementers of EE practices and to reach and motivate the millions of farmers in Vietnam, two TV serials developed using entertainment-education principles were launched to promote flower growing and reducing insecticide use. The TV serials helped farmers "see" and appreciate the role of parasitoids by linking the lesser known parasitoids (known locally as "small bees") to better known bees. Farmers that viewed the serials decreased their insecticide use by 24%, had 3.3% increased yields, increased their appreciation of parasitoids and gained positive attitudes towards the growing of flowers.

To achieve area-wide sustainable pest management, EE practices have to be coupled with rational pesticide management through better pesticide policies, regulations and implementation, accurate pest diagnostics and timely professional advice to farmers. Increasing biodiversity and ecosystem services in rice fields would also contribute towards climate change adaptation.

Abstract ID 273

Exclusion, Suppression, and Eradication of Pink Bollworm (*Pectinophora gossypiella* (Saunders)) from the Southwestern USA and Northern Mexico

Eoin DAVIS

US Department of Agriculture (USDA), USA, Email: *eoin.b.davis@aphis.usda.gov*

Pink bollworm (PBW) is an economically important invasive species to North America, first detected in the USA in 1917 in the State of Texas. By 1965 it had spread west and was a major economic pest across the southwestern US and northern Mexico.

The USDA and the California cotton industry launched a Sterile Insect Technique (SIT) programme in 1967 to suppress PBW in the large central valley of California, and to prevent further northward spread.

The success of that effort and the combination of SIT, effective mating disruption technology, Bt-cotton, cultural control, and intensive trapping and mapping allowed a highly successful area-wide eradication programme to begin in 2001/2002.

As of the end of 2016, no PBW have been detected in the eradication area in the southwestern US and northern Mexico for nearly 5 years.

Abstract ID 226

Use of Pheromones to Disrupt Mating of Moth Pests in Area-Wide Management Programmes

Ring CARDÉ

University of California, Riverside, California, USA, Email: *ring.carde@ucr.edu*

Disruption of mate finding by broadcast application of formulated, synthetic pheromone is a well-established method for control of many moth pests. There are three principal mechanisms considered responsible for mating disruption: sensory impairment of males, competition between point sources of formulated pheromone and calling females, and a camouflage of the female's pheromone plume by omnipresence of synthetic pheromone. The relative contribution of these mechanisms to efficacy of disruption varies with formulation type, application rate, and behavioural characteristics of the pest species.

A major determinant of success, however, can be the rate of immigration of mated females into treated areas. Therefore, generally either the larger the area under treatment or the more isolated it is from sources of infestation, the greater the probability of successful disruption.

How disruption mechanisms, formulation types, and the size and isolation of treatment plots interact to influence efficacy is reviewed for the codling moth *Cydia pomonella*, Oriental fruit moth *Grapohlita molesta*, European grape vine moth *Lobesia botrana*, pink bollworm *Pectinophora gossypiella*, navel orangeworm *Amyelois transitella*, and gypsy moth *Lymantria dispar*.

Finally, implementing mating disruption in area-wide programmes requires close coordination and cooperation between governmental and grower groups and integration with other management techniques.

Abstract ID 278

Holistic Area-wide Approach for Successfully Managing Citrus Greening (Huanglongbing) in Mexico

Francisco Javier TRUJILLO-ARRIAGA and Clemente de Jesús GARCÍA-AVILA

Dirección General de Sanidad Vegetal del SENASICA, Ministry of Agriculture, México City, Mexico, Email: *clemente.garcia@senasica.gob.mx*

The Plant Health General Directorate (DGSV in Spanish) is recognized as the National Plant Protection Organization of the Federal Government of Mexico that acts under the Plant Health Federal Law. Some relevant plant protection programmes that Mexico is implementing include: The Huanglongbing (HLB) - Asian Citrus Psyllid (ACP) Programme, Mediterranean Fruit Fly Programme, National Fruit Fly Campaign, Pink Hibiscus Mealybug Programme and a permanent Phytosanitary Surveillance Programme to prevent the introduction and spread of regulated non-native pests.

HLB or citrus greening is caused by the bacterium *Candidatus Liberibacter* spp., considered the most devastating disease in the world of citriculture. It causes the death of orange, mandarin, grapefruit and lemon plants in 3 to 8 years, once infected. The HLB is transmitted by the ACP (*Diaphorina citri* Kuwayama), an insect vector widely distributed in the citrus producing regions of the world, including in the citrus areas of Mexico and the Americas. Until 2004 the disease only existed in Asia and Africa; in 2005 it was detected in Florida, USA. The HLB disease was detected in 2009 in Yucatán, Mexico. In this year the citrus production was 6.82 million tons (SIAP, 2015). The economic impact evaluation by Salcedo et al. (2010) indicated that, without the intervention of the Federal Government, in five years with HLB, the Mexican citrus production would be reduced by 2.7 million tons (39.6%). However, in 2015, the citrus production in Mexico was 7.57 million tons (SIAP, 2015).

Mexico has 572,000 hectares of citrus and only 9.4% have HLB disease. The first phytosanitary actions implemented on an area-wide basis were: timely detection of HLB in citrus and urban areas, elimination of infected plants in areas under control, control of the ACP vector and protection of propagating material (nurseries). As a result of the successful HLB Programme, since its appearance in 2009 the disease has been contained, and over 90% of citrus areas in Mexico are still free of HLB disease.

Management of HLB now is organized through Regional Areas of Control (ARCOS), which implement the following area-wide measures: Criteria associated to climate, host presence in urban or cultivated areas, monitoring psyllids, chemical control, and biological control. From 2010 to 2015, 31 billion parasitoid wasps *Tamarixia radiata* were produced and released in in citrus production and abandoned, urban and backyard areas in Campeche, Chiapas, Guerrero, Hidalgo, Oaxaca, Quintana Roo, Tabasco, and Yucatan States. The ARCOS are public-private organizations that are jointly operated by government federal and citrus growers.

In 2016 the Mexican government assigned an annual budget of almost US $8.5 million to the HLB Programme. With these actions, Mexico has prevented the rapid spread of the disease to new areas and significantly impacted populations of the ACP. In addition, research programmes have been generated through scientific institutions to produce vegetative material with tolerance or resistance to the disease. Although the government has successfully implemented area-wide strategies for regional control, it is necessary to develop new improved technologies to eliminate the vector, following the example of the Mediterranean fruit fly Programme in Mexico.

Abstract ID 270

Area-wide Biological Control Using the Parasitic Natural Enemy *Aphidius gifuensis* (Hymenoptera: Braconidae) to Manage *Myzus persicae* (Homoptera: Aphididae) in China

Hailin YANG and Yanbi YU

Yunnan Agricultural University, Kunming, Yunnan, China, Email: *3641775@qq.com269*

The biological control of aphids (*Myzus persicae*) using the parasitoid natural enemy *Aphidius gifuensis*, is being applied in large areas of China after 20 years of research. Two sets of efficient, economic, simple techniques of mass breeding parasitoids: "*adult plant breeding method*" and "*seedling breeding method*" have been created, which are suitable for different planting regions.

"*Collecting-Storage-Transport-Releasing*" techniques, as well as the parasitoid collection and release devices, have also been invented. They contribute significantly to highly efficient automatic gathering and accurately releasing of *Aphidius gifuensis*.

The use of the "*unified technical standard, zoning classification control*" technique and "*one mainstay with multivariate, one project with double propel methods*" mode achieved the extensive usage on flue-cured tobacco, vegetables, oilseed rape, broad bean and other kinds of crops. In this mode the company is the mainstay of technology application, together with local governments, farm owners and other organizations. The research projects are major forces of implementation, with technical training and testing to consolidate the effects.

From 2010 to 2016, cumulatively, these techniques had been applied in 60.28 million mu (1 mu is about 0.07 hectare), including 42.95 million mu of flue-cured tobacco, with a control effect of 60%-92%, and 17.33 million mu of other crops with a control effect of 39%-65%. It has become the most important and the most widely applied green control technique of pest control on tobacco agriculture in China.

The application of these techniques have reduced by more than 3682 tons the application of chemical pesticides, saved 2.852 billion yuan of control costs, recouped 16.44 billion yuan of economic losses, and benefited more than 1 million peasant households. The way of pest control in the main tobacco production area in China has been transformed from chemical control into biological control. Appling area-wide the biological control technique turned the strategy to manage aphids from "passive response" into "active attack". Because of the significant benefit to the economy, society and ecosystem, it gained a great recognition from academicians of the Chinese Academy of Sciences and the Chinese Academy of Engineering, as well as from international experts of biological control from Japan, USA and other countries.

Abstract ID 142

Putting Sterile Insect Technique into the Modern IPM Toolbox: Over 20 Years of Successful Area-wide Integrated Pest Management in Canadian Pome Fruit

Cara NELSON, Evan ESCH and Melissa TESCHE

Okanagan Kootenay Sterile Insect Release Program, BC, Canada, Email: *cnelson@oksir.org*

Background: The Sterile insect technique (SIT) has been successfully used for decades to control or eradicate numerous pests in a diversity of crop and habitats across the globe. Despite this proven success, SIT is often considered a curiosity rather than an effective, environmentally-friendly technology that dovetails into many modern integrated pest management (IPM) programmes.

Methodology: The Okanagan-Kootenay Sterile Insect Release (OKSIR) Programme has conducted a successful area-wide IPM programme in southern British Columbia (BC), Canada for over 20 years. Here, SIT is the primary tool that controls the key pest of pome fruits in the region, the codling moth, *Cydia pomonella* (L.). Chemical, cultural, and biological techniques that complement SIT are also used as needed. Our SIT programme is supported by close monitoring of codling moth populations in orchards and adjacent urban properties; enforcing suppression of codling moth infestations in urban areas; removal of derelict orchards, wild host trees, and poorly managed host trees in urban areas; and increased public awareness and education.

Results: Our programme results are impressive. Successful collaboration between the OKSIR programme, the pome fruit industry, area residents, and various government organizations have reduced codling moth populations by 94% and codling moth damage to <0.2% of fruit damaged in >90% of the orchards in the programme area. Local pesticides sales data estimate a 96% reduction in amount of active ingredient used against the codling moth since 1991. The OKSIR programme exemplifies the effectiveness of SIT as part of a modern, area-wide IPM programme.

As globalization makes relative distances between regions smaller, opportunities to leverage existing SIT infrastructure increase. The codling moth rearing facility in Osoyoos, British Columbia, Canada has the capacity to produce 780 million sterile codling moths annually, only a part of which is used seasonally to treat 3,400 hectares of pome fruit made up of small orchards intermixed with residential areas in the Okanagan Valley. The OKSIR Programme is exploring opportunities to diversify its business model to provide added value to local stakeholders by taking advantage of the unique governance structure of this community-based area-wide IPM approach.

Conclusion: Today, destructive insect pests are migrating to new habitat throughout the world as a result of climate change and global trade. These new threats must be managed in ways that protect both the agri-food industries, and the natural environments in which the industries operate. The OKSIR Programme stands out as a highly effective and easily transferrable model to meet these challenges. Indeed, OKSIR is one of the very few IPM programmes in the world that is able to combat infestations across multiple jurisdictions, using environmentally-friendly, cost-effective methods that are based on proven technology.

Abstract ID 76

The Suppression of the False Codling Moth, *Thaumatotibia leucotreta* in South Africa using an AW-IPM Approach with a SIT Component

Nevill BOERSMA

XSIT, Citrusdal, South Africa, Email: *nb@xsit.co.za*

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is native to sub-Saharan Africa and infests various commercial and wild fruit-bearing plants. The insect has phytosanitary implications for the United States of America, the East and possibly Europe, which imposes a huge risk on exports, conveying economic threats to both the fruit industry of South Africa and the growers. This may have a socio-economic impact on both food and job security. Although the pest was controlled to some extent by measures of insecticides, mating disruption and orchard sanitation, a longer term solution was needed in view of increasing insecticide resistance.

Research on an area-wide IPM programme, in conjunction with the release of sterile insects, started in 2002. Commercial sterile insect releases over 1 500 ha of citrus orchards in the Citrusdal region started in 2007, expanding to almost 20,000 ha in three different regions in 2017.

Over the past ten years the status of *T. leucotreta* as a pest threat was systematically reduced in areas where sterile insects were released, compared to non-release areas.

Abstract ID 286

Advances in Integrated Tick Management for Area-wide Mitigation of Tick-borne Disease Burden

Adalberto A. PÉREZ DE LEÓN

USDA-ARS Knipling-Bushland U.S. Livestock Insects Research Laboratory, and Veterinary Pest Genomics Center, Kerrville, Texas, USA, Email: *Beto.PerezdeLeon@ARS.USDA.GOV*

Ticks and Tick-borne diseases (TTBD) burden the health of humans, domestic animals, and wildlife, and the productivity of livestock worldwide. Global change exacerbates the problems with TTBD. Some high-consequence TBD are transmitted by invasive tick species that have become resistant to chemical control, the involvement of wildlife can complicate TTBD management programmes, and challenges with TTBD can have a transboundary dimension. Integrated approaches are required to mitigate the burden of TTBD.

Hypothesis-driven, science-based research is enabling the innovation of strategies to address the complex problem of TTBD in a sustainable manner (Figure 1). The adaptation of vaccines, acaricidal essential oils, biocontrol agents, and enhanced delivery systems for use in combination with other technologies is advancing integrated tick management (ITM) research efforts.

Public-private partnerships play a crucial role to translate research into efficacious products approved by, or registered with regulatory agencies that can be utilized safely by end-users according to label instructions. ITM research in Puerto Rico delivered protocols that decreased significantly morbidity and mortality due to bovine babesiosis in dairy cattle herds. A public-private partnership resulted in the integrated use of a Bm86-based vaccine by the U.S. Cattle Fever Tick Eradication Programme.

The application of robotics, remote sensing technology, and genomics to address research questions on TTBD is opening exciting possibilities to improve area-wide ITM programmes. This provides context for the One Health approach to deal with TTBD of veterinary and public health importance.

Fig. 1. *Suggested research framework toward sustainable integrated tick and tick-borne disease management strategies in the context of global change and the One Health approach.*

*Adapted from Pérez de León AA, Teel PD, Auclair AN, Messenger MT, Guerrero FD, Schuster G, Miller RJ. 2012. Integrated strategy for sustainable cattle fever tick eradication in USA is required to mitigate the impact of global change. Front Physiol. 3:195. doi: 10.3389/fphys.2012.00195.

Abstract ID 255

Area-wide Fruit Fly Programmes in Latin America

Pedro RENDÓN[1] and Walther ENKERLIN[2]

[1]Programa Moscamed, Guatemala, Email: *pnodner@hotmail.com*
[2]FAO/IAEA, Vienna, Austria

Background: The Latin-American region (including the Caribbean - LACAR) with a surface area of ca. 19.2 million km^2 and > 626 million inhabitants, has a variety of climatic conditions apt for the production of fruits and vegetables for local consumption and export. In fact, some countries in the region are amongst the major producers/suppliers of produce worldwide. Despite their production potential, countries in LACAR see trade possibilities reduced or production/investments threatened by the presence of native or non-native fruit flies of the Tephritidae family.

Native to the LACAR are the genera *Anastrepha* and *Toxotrypana*, the former includes seven economically important species that are pest problems to a reduced number of fruits (i.e. species are more host specific), while the latter is known to attack papaya.

In the American continent, one classic example of a high-profile non-native pest introduction is the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), which was detected in 1901 in Brazil and has since infested other countries in South and Central America putting at risk more than 300 species of fruits and vegetables and hampering commercial production and exports worth millions of dollars/year. Other species of the Tephritidae that have also been introduced/established in some parts of the region are *Bactrocera carambolae* and *B. oleae*.

Currently, one major concern is the potential risk of introductions of fruit fly species of economic and quarantine importance not present in any of the countries of the region, which could later lead to wide-spread invasion due to their reproductive potential, and the lack of natural enemies and expertise for control.

Methods: For the past 45 years the IAEA - Technical Cooperation (TC) and the Insect and Pest Control Section of the Joint FAO/IAEA Division have collaborated with Member States in LACAR in transferring area-wide integrated fruit fly management technology, including in some situations the sterile insect technique (SIT). Through this mechanism of cooperation, a number of large-scale operational programmes have been established and consolidated such as programmes in Argentina, Belize, Chile, Guatemala, Mexico and Peru. Support has also been provided to smaller scale programmes aimed at establishing specific areas for production and exports, for example, in Central America, as well as a programme to eradicate a large Mediterranean fruit fly outbreak in Dominican Republic.

More recently, a regional project is ongoing aimed at strengthening surveillance systems and emergency response capacity against new non-native fruit flies and continuing the support to

the expanding fruit fly low prevalence or free areas which are conducive for fruit and vegetable production and exports.

Results and Conclusion: Due to the unavoidable increase in human travel, global trade, changes in climatic conditions and the existence of threatening invasive transboundary insect pests, the area-wide approach to early detect and control pest at country and regional level has shown to be a more cost-effective and environmentally friendly option compared with the individual reactive farm by farm approach. This can be verified by the huge return on investment accrued by the area-wide programmes, some of which integrate the SIT, that have protected the well-being of the horticultural industries and fostering development and further expansion in many LACAR countries. The contributions and leading role of the IAEA and FAO through the transfer of area-wide SIT technology are relevant to the food security and sustainable development goals of the countries in the region.

Abstract ID 48

Area-wide Management of Fruit Flies in a Tropical Mango Growing Area Using the Sterile Insect Technique: From a Research Project to an Operational Programme

Pablo LIEDO[1], Pablo MONTOYA[2] and Jorge TOLEDO[1]

[1]El Colegio de Frontera Sur (ECOSUR), Tapachula, Mexico, Email: *pliedo@ecosur.mx*
[2]Programa Moscafrut, SAGARPA, IICA, Tapachula, Mexico

Background: The Sterile Insect Technique (SIT) has been successfully used for the control of fruit flies in a number of places in the world. One requirement for its successful application, is that wild populations should be at low densities. This has been one important reason that has prevented its application in tropical fruit growing areas, where climate conditions and the availability of hosts all year round result in high population densities.

Here we report the results of a project where SIT integration was evaluated under the tropical conditions of the mango growing area in the Soconusco region, in Chiapas, Mexico. The basis for the area-wide integrated management approach was the knowledge on the population dynamics of pest fruit flies in the region. The main mango growing areas are located in the coastal lowlands, where fruit fly populations tend to be very low outside of the mango season. Population densities are higher at mid-elevations and highlands, where alternate hosts are common in back-yards and as part of the natural vegetation, which we call refugia areas.

Background: The area-wide management strategy consisted in establishing a biological barrier with releases of parasitoids and sterile flies to prevent or minimize the dispersal of wild flies from the refugia areas to the mango orchards.

Results: After two years of releases, population densities were suppressed over 70% in the area of releases and around 65% in the whole area (including the lowlands where mango orchards are located).

With the support of fruit growers, and state and federal governments, this project was continued as an area-wide integrated management operational programme. In 2016, after 4 years, the detection of wild flies was significantly reduced, and the number of batches of fruit that were rejected at the packing houses due to the detection of infested fruits was the lowest in the past 12 years, since these data have been recorded.

Conclusion: These results demonstrate that area-wide management with SIT can be applied successfully under tropical conditions with natural high densities of fruit flies, provided there is knowledge on the fruit fly population dynamics as a basis to design the area-wide management strategy.

Session 2: Mosquitoes and Human Health

Abstract ID 289

WHO Vector Control Advisory Group Activities and WHO Vector Control Global Response 2017-2030

Raman VELAYUDHAN

Vector Ecology and Management Unit, Department of Control of Neglected Tropical Diseases (HTM/NTD), World Health Organization, Geneva, Switzerland, Email: *VelayudhanR@who.int*

WHO Vector Control Advisory Group

The Vector Control Advisory Group (VCAG) was constituted in 2013 by the World Health Organization (WHO) to advise on the efficacy of new tools, technologies and approaches for public health vector control. VCAG issues advice to WHO to inform policy recommendations as well as to innovators of new vector control interventions to guide product development. To date, VCAG has assessed a number of new product classes for vector control with diverse entomological modes of action, which aim to reduce the transmission and burden of vector-borne diseases in humans. VCAG advice on new interventions will include:

- early interaction with investigators on the submission to clarify if it constitutes a new product class and/or product claim;
- VCAG initial concept review and determination of data generation required in order to substantiate a new product class or product claim; and
- VCAG final review of evidence, including (a) in the case of a new product class, completion of the target product profile (TPP) and (b) validation of that claim.

WHO Vector Control Global Response 2017-2030

Vector-borne diseases pose a major threat to the health of societies around the world. They are caused by parasites, viruses and bacteria transmitted to humans by mosquitoes, triatomine bugs, blackflies, sandflies, ticks, tsetse flies, mites and lice. The major global vector-borne diseases of humans include malaria, dengue, lymphatic filariasis, chikungunya, onchocerciases, Chagas disease, leishmaniasis, Zika virus disease, yellow fever and Japanese encephalitis. Other vector-borne diseases, such as human African trypanosomiasis, Lyme disease, tick-borne encephalitis and West Nile fever, are of local importance in specific areas or populations.

The major vector-borne diseases together account for around 17% of the estimated global burden of communicable diseases and claim more than 700 000 lives every year. The burden is highest in tropical and subtropical areas. More than 80% of the global population lives in areas at risk from at least one major vector-borne disease, with more than half at risk from two or more. The risk of infection for certain viral pathogens is particularly high in towns and cities where *Aedes* and *Culex* mosquitoes proliferate, because of favourable habitats and close

contact with human beings. Impressive gains have been made against malaria, onchocerciasis, lymphatic filariasis and Chagas disease, but the burden of many other vector-borne diseases has increased in recent years.

Since 2014, major outbreaks of dengue, malaria, chikungunya and yellow fever have afflicted populations, claimed lives and overwhelmed health systems in many countries. Zika virus infections and their associated complications rapidly spread across the WHO Region of the Americas and beyond in 2016, affecting individuals and families and causing social and economic disruption. In order to address these challenges and based on the request of member states the draft *Global Vector Control Response 2017–2030* was developed through an extensive consultation process that began in June 2016 with the aim of adoption of the response by the Seventieth World Health Assembly in May 2017.

Abstract ID 220

Area-Wide Mosquito Management in the Americas

Graham WHITE

University of Florida, Gainesville, Florida, USA, Email: *gbwhite@ufl.edu*

Vector-borne diseases (VBD) transmitted by mosquitoes were endemic in all 47 countries of the Americas. Area-wide (AW) sanitation campaigns began in Cuba in 1900 to suppress malaria and yellow fever (YF), progressed to facilitate construction of the Panama Canal and the new Panamax is virtually free from VBD risk.

From 1950s, nation-wide house-spraying with DDT and then other insecticides (organophosphates, carbamates, pyrethroids) against *Anopheles* malaria vectors, coupled with improved mosquito-proofing of houses, eliminated malaria from North America and cut the areas of risk to foci in <20 countries: incidence <400,000, mortality <80 in 2015. Human filariasis has been eliminated by controlling the vector *Culex* in all but 2 countries.

Efforts to eliminate *Aedes aegypti*, the invasive vector of YF, dengue and recently chikungunya and Zika, were successful in 22 countries during 1960s, followed by serious resurgence; now several genetic approaches, including the sterile insect technique (SIT), are being developed to augment environmental and insecticidal control of *Ae. aegypti*.

Most Member States of the Pan American Health Organization (PAHO) have Health Ministry run strategies and operations for Integrated Vector Management (IVM), delegated to States and Municipalities for operational implementation. The USA has decentralized mosquito control operations implemented by locally organized districts and counties in each State, officially advised by the Centers for Disease Control and Prevention (CDC) and coordinated unofficially by the American Mosquito Control Association (AMCA), with emphasis on AW reduction or prevention of seasonal mosquito problems from marshlands.

Commercial contractors [pest control operators (PCOs) and the National Pest Management Association (NPMA)] play important roles for mosquito control in the USA, for clients at all levels of society, with considerable potential for AW mosquito control contracts, especially recent experiences of contractors with fleets of aircraft and trucks being hired to combat outbreaks of dengue in Dallas (2015) and Zika in Miami (2016).

This presentation gives case-studies and discusses the IVM scope for more AW interventions under management systems currently practiced in various countries of the Americas.

Abstract ID 279

Malaria Elimination in Sri Lanka

Rajitha WICKREMASINGHE

Faculty of Medicine, University of Kelaniya, Kelaniya, Sri Lanka, Email: *arwicks@sltnet.lk*

Malaria was endemic in Sri Lanka for centuries. Organised malaria control activities started with the establishment of the Anti-Malaria Campaign in 1911. There was a massive epidemic of malaria in 1934/35 which resulted in 1.5 million cases and an estimated 80,000 deaths in a 9-month period when the population of the country was 5 million. Prior to the DDT era which commenced in 1945, malaria control activities focused on larviciding and distribution of antimalarial medicines.

The introduction of DDT as a residual insecticide spray met with immediate success. In 1957, Sri Lanka launched a malaria eradication programme. In 1963, only 17 cases of malaria were reported in the country, of which only 6 cases were due to indigenous cases. With this achievement, indoor residual spraying was reduced. Indigenous transmission of malaria was continuing and Sri Lanka experienced another major epidemic of malaria in 1967-69 when about 1.5 million cases were reported.

With this setback, Sri Lanka moved into malaria control mode of operation. Malathion was introduced in 1975, and by 1977, all malarious areas were sprayed with Malathion. After an initial reduction of the number of malaria cases, there was another epidemic of malaria in 1986 when more than 600,000 cases were reported. Sri Lanka adopted the revised malaria control strategy of the WHO in 1993.

In 1999, more than 250,000 cases of malaria were reported. Sri Lanka then adopted a strategy of conducting mobile malaria clinics in which teams went into the community to actively detect and treat cases. By 2001, there was a dramatic reduction of cases and by 2008 there was a reduction of over 99% of cases as compared to 1999.

By 2004, Sri Lanka had achieved the WHO criterion to launch the malaria pre-elimination phase, but chose not to do so, given the prevailing separatist war in the north and east of the country which were endemic for malaria.

In 2009, Sri Lanka embarked on the malaria pre-elimination phase with Global Fund to fight AIDS, Tuberculosis and Malaria (GFATM) funding. The objective of the programme was to eliminate *P. falciparum* malaria by the end of 2012 and *P. vivax* malaria by the end of 2014, focusing on intensified surveillance.

In 2010/2011 Sri Lanka commenced the malaria elimination phase and the last indigenous case of malaria was reported in October 2012. Since then only less than 100 cases of imported malaria have been reported per year in the country.

The majority of imported malaria cases have been among Sri Lankans who acquired the infection during travels overseas, especially to countries in the region, mainly India. Cases have also been recorded among foreign nationals some of whom were refugees. High risk groups include Sri Lankan armed forces personnel serving UN peace keeping missions, pilgrims and traders to India, businessmen travelling to Africa for fishing and gem mining, illegal migrants and returning refugees from India. All these populations are screened for malaria on arrival.

Sri Lanka was certified malaria-free by the WHO in September 2016.

Abstract ID 250

City-wide Control of *Aedes aegypti* and Zika Virus Using Mass-trapping in Puerto Rico

Roberto BARRERA

US Centers for Disease Control and Prevention, San Juan, Puerto Rico, USA, Email: *rbarrera@cdc.gov*

Background: Aedes aegypti is the main vector of dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) viruses in Puerto Rico and the Americas. This species has become resistant or partially resistant to most pyrethroid insecticides tested so far in Puerto Rico.

Non-insecticidal mosquito traps were initially tested in small, isolated communities in Puerto Rico to determine if they caused significant and sustained area-wide reductions of *Ae. aegypti* populations, viruses in mosquitoes, and human cases. With adequate numbers of traps per home and spatial coverage (>80% of homes), Autocidal Gravid Ovitraps (AGO) significantly and persistently reduced *Ae. aegypti* density, CHIKV prevalence in mosquitoes and human cases in 2014, when this virus invaded the islands. No local outbreaks of CHIKV were observed in intervention areas with mosquito densities below a threshold of 3 females / trap / week.

In this presentation, we report on a scale-up intervention in a city with 100,000 inhabitants using an integrated vector control approach, consisting of source reduction, larviciding and 3 AGO traps/home during the 2016 Zika epidemic in Puerto Rico. The main goal was to reduce *Ae. aegypti* densities below the identified threshold.

Methodology: An area with 30,000 houses in Caguas City was divided into eight contiguous zones, which were randomly and sequentially treated (cluster randomized stepped wedge design) by visiting buildings to conduct source reduction, larviciding (*Bacillus thuringiensis israelensis*), and installing three AGO traps.

The study began in October 2016 and is ongoing. Mosquito densities in all 8 zones were monitored every week using 360 sentinel AGO traps. Mosquito pools per trap per week were processed by RT-PCR to identify RNA of DENV, CHIKV, and ZIKV. A Geographical Information System was used to keep track of vector control interventions and trap placement.

Results: Pre-intervention densities of *Ae. aegypti* in all zones were above threshold levels and fluctuated widely as a result of rainfall fluctuations. Twenty eight ZIKV and one DENV virus positive pools of *Ae. aegypti* were found in the first seven weeks of the study. Significant vector reductions have begun to be observed in the areas that were treated first.

Conclusion: Keeping track of vector control coverage in the different zones is perhaps the most important task to be accomplished during implementation. Difficulties found during implementation of this scale-up intervention will be discussed.

Abstract ID 104

Developing the Combined IIT/SIT Approach to Control the Primary Dengue Vector *Aedes albopictus* in China

Zhiyong XI[1], Ziqiang YAN[2] and Xiaoying ZHENG[3]

[1]Sun Yat-sen University, Michigan State University Joint Center of Vector Control for Tropical Diseases, Guangzhou, China, Email: *xizhiyong@yahoo.com*
[2]Guangzhou Center of Disease Control and Prevention, Guangzhou, China
[3]Sun Yat-sen University, Guangzhou, China

The endosymbiotic bacterium *Wolbachia* is widely recognized for its ability to induce both a reproductive abnormality known as cytoplasmic incompatibility (CI) and a resistance to dengue virus in mosquitoes. The *Wolbachia*-based population suppression strategy, referred to as Incompatible Insect Technique (IIT), entails the release of male mosquitoes infected with *Wolbachia*, resulting in sterile matings and a reduction in the mosquito population.

Here, we will report an ongoing field trial to control dengue mosquito vectors through the release of males to induce female sterility in Guangzhou China. The released *Aedes albopictus* HC strain carries a novel *Wolbachia* from *Culex pipiens* mosquito, which induces both CI toward the wild type mosquito and resistance to dengue virus.

Mass-rearing capacity has been successfully developed with a production of >5 million males per week. An X-ray irradiator has been developed for irradiating the mass-produced pupae to prevent the potential of population replacement caused by the released females escaped from the sex separator.

Significant suppression of the mosquito populations has been accomplished in the release sites compared to the control. We will discuss our results in relation to implementation of SIT/IIT for the area-wide suppression of mosquito population for disease control.

Abstract ID 160

Area-wide Management of *Aedes aegypti* Mosquito Vector: The First Pilot Suppression Trial in Thailand Integrating the Sterile Insect Technique with *Wolbachia*-induced Incompatibility Approach

Pattamaporn KITTAYAPONG[1,2], Ninphanomchai SUWANNAPA[1], Supaluk KHAKLANG[1], Wanitch LIMOHPASMANEE[3], Chitti CHANSANG[4], Uruyakorn CHANSANG[4] and Piti MONGKALANGOON[4]

[1]Centre of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University at Salaya, Nakhon Pathom, Thailand, Email: *pkittayapong@gmail.com*
[2]Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand
[3]Thailand Institute of Nuclear Technology, Ministry of Science and Technology, Nakhon Nayok, Thailand
[4]Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Background: Dengue, chikungunya and Zika disease, mainly transmitted by *Aedes aegypti* mosquitoes, are important public health problems in Thailand and worldwide. Currently there are no drugs or effective vaccines available to treat these diseases and vector control is the only method for disease prevention.

Our study aimed to provide proof-of-concept that area-wide vector management integrating sterile insect technique with *Wolbachia*-induced incompatibility approach could suppress natural *Ae. aegypti* populations.

Methodology: Direct microinjection was used to generate an *Ae. aegypti* colony that is double-infected with *Wolbachia* strains obtained from *Ae. albopictus*. Laboratory experiments were performed to test sterility and mating competitiveness of the developed *Ae. aegypti* line after pupae were irradiated at 50 Gy and 70 Gy. One-year data on household abundance and egg hatch rate of *Ae. aegypti* mosquitoes collected from the study site were used to form the baseline for monitoring the release of the super-sterile males.

Results: In this study, a *Wolbachia*-transinfected *Ae. aegypti* line (THAB) was successfully established. Complete sterility of both males and females was observed when they were irradiated at 70 Gy during the pupal stage. Mating competitiveness between super-sterile males and normal ones demonstrated that the appropriate release ratio was 10:1.

The pilot field trial was attempted for the first time in an ecologically isolated village of 140 households in Plaeng Yao District, Chachoengsao Province, eastern Thailand. Based upon the baseline data, an estimate of 10 *Ae. aegypti* female mosquitoes per house was used to determine the required number of release. Hundred super-sterile males, held in a screened plastic container containing 10% sugar solution, were then released each week into each household using local public health volunteers.

After 16 weeks of field monitoring, our results from ovitraps confirmed significant reduction in the egg hatch rate ($p<0.05$) of natural *Ae. aegypti* populations at the treatment village when compared to the control one. Community engagement and public awareness through media resulted in positive support for practical application of this strategy in wider areas.

Conclusion: Our pilot field trial demonstrated the first proof-of-concept that natural *Ae. aegypti* populations could be suppressed using an area-wide vector management integrating sterile insect technique with *Wolbachia*-induced incompatibility approach.

Abstract ID 267

Wolbachia-Aedes - an Additional Tool to Control *Aedes aegypti* in Singapore?

Lee Ching NG

National Environment Agency, Environmental Health Institute, Singapore and WHO Collaborating Centre, Singapore, Email: *NG_Lee_Ching@nea.gov.sg*

Background: For more than 50 years, Singapore has taken a multipronged approach in tackling the challenge of dengue. Community and inter-sectoral participation, coupled with legislation support the elimination of *Aedes* breeding habitats, which is vital for the reduction of *Aedes* population in the community.

In the face of an increasing challenge, the *Aedes* control programme has evolved through the decades, including through the incorporation of science and technologies such as data analytics for risk forecast and stratification, development of Gravitrap for entomological surveillance and molecular biology for virus surveillance.

Methods: Male *Wolbachia-Aedes* is yet another tool under development to further suppress the urban *Aedes aegypti* in the community. When the released male *Wolbachia-Aedes* mate with urban female *Aedes aegypti* in the community, cytoplasmic incompatibility should thwart the hatching of their resultant eggs, thus leading to no progenies. After 4 years of laboratory studies, risk assessment and stakeholders consultation, *Project Wolbachia-Singapore* reached a significant milestone when male *Wolbachia-Aedes* were brought into field studies in October 2016.

Results: Intensive community engagement has led to very strong support from stakeholders and the community. Following 12 weeks of releases at Tampines West site, the hatchability of *Aedes* mosquito eggs collected from the site has indeed shown a reduction. The data suggest that the males *Wolbachia-Aedes* produced by *Project Wolbachia-Singapore* are competitive in the field.

Conclusions: This is part of the small scale field study that involved releases of male *Wolbachia-Aedes aegypti* mosquitoes at three selected study sites located within Braddell Heights, Nee Soon East and Tampines West, to further understand their behaviour in the urban built-up environment. Besides demonstrating their competitiveness, we have also obtained useful data that will be used as input in mathematical models, which will guide our follow-up suppression trial.

Abstract ID 222

Developing AW-SIT as Part of an Integrated Control Strategy against *Aedes sticticus* in the River Dalälven Floodplains, Central Sweden

Jan LUNDSTRÖM[1,2], Eric BLOMGREN[1], Martina SCHÄFER[1] and Pattamaporn KITTAYAPONG[3]

[1]Swedish Biological Mosquito Control, NEDAB, Uppsala, Sweden, Email: *jan.lundstrom@mygg.se*
[2]Zoonosis Science Center, Medical Biochemistry & Microbiology, Uppsala University, Uppsala, Sweden
[3]Centre of Excellence for Vectors & Vector-Borne Diseases, Faculty of Science, Mahidool University at Salaya, Nakhon Pathom, Thailand

The floodwater mosquito *Aedes sticticus* is a nuisance species utilizing temporary flooded wetlands as larval habitat. Females disperse 5-10 km from the 100 km^2 of temporary wetlands of the River Dalälven floodplains, causing nuisance in 10,000-40,000 km^2 of upland areas. Control of mosquito larvae using VectoBac G is efficient in reducing the nuisance, and is highly appreciated by local people, tourists and other visitors. However, the Government is concerned about the sustainable use of VectoBac G.

In 2015, the Swedish Biological Mosquito Control/NEDAB was required to propose an alternative control method, and in 2016 the requirement was for a plan to reduce use. The recent progress in adapting the Area-Wide Sterile Insect Technique (AW-SIT) approach for controlling *Aedes aegypti* and *Aedes albopictus* indicate a potential for adapting SIT for controlling other *Aedes* mosquitoes. The potential usefulness of applying the SIT, as part of an integrated control strategy, against *Ae. sticticus* needs to be evaluated for "proof-of-concept" in a pilot study, before any evidence-based decisions could be made.

The ecology of *Ae. sticticus* makes it a potentially suitable target species for SIT application. The whole blood seeking female population, causing nuisance in a very large surrounding area, have their larval and mating habitats in small and well defined wetlands. Release of sterile males can therefore be highly focused, greatly increasing speed and reducing costs. With respect to the flight range of *Ae. sticticus*, we selected study sites with no nearby production of the species, and with all known production sites within flight range included in the mosquito control programme. Within the selected pilot study sites the VectoBac G based control have reduced the target population dramatically providing a population size suitable for control by introducing the sterile males.

Important tools for evaluating an SIT study are methods for measuring the abundance of males, females, larvae and eggs of the target species. Male sampling is often the most difficult, while female and larval sampling are established techniques for *Ae. sticticus*. In addition, we have shown that electrical aspirators can sample the males, and we are setting up methods for measuring the abundance of eggs.

The release of sterile male *Ae. sticticus* for the pilot study requires a laboratory colony of the target species, timely and reliable production of sterile males, and methods for male transportation and release. We have established a laboratory colony and they are mating in confined space and laying eggs on artificial substrate. Our *Ae. sticticus* colony completes the cycle from flooding of eggs over all life stages to the next generation of eggs in 11 days at 21^{o}C.

The sterile male *Ae. sticticus* needed for the pilot study are suggested to be produced in Bangkok, allowing delivery by flight to the Swedish study site in about 15 hrs. This is a promising and challenging project, with potential to break ground for a new, efficient, sustainable and environmentally safe control method against floodwater *Aedes* mosquito species in natural wetland environments.

Abstract ID 287

Aedes aegypti Control Programmes in Brazil

Danilo O. CARVALHO[1], Luiza GARZIERA[2], Michelle C. PEDROSA[2], Iana LIMA[2], Jair F. VIRGINIO[2] and Margareth CAPURRO[1]

[1]Universidade de São Paulo, Brazil, Email: *mcapurro@icb.usp.br*
[2]Moscamed Brazil Facility, Juazeiro, Brazil

Background: In 2016, the Brazilian Health Ministry reported more than 1.5 million cases of dengue and 215 thousand cases of Zika. According to the entomological surveillance, around 37.5% of the cities are under alert or risk regarding the *Aedes* mosquito population density. In order to control the vector and disease transmission, Brazil has developed the *National Plan of Control of Dengue* (PNCD), which develops permanent programmes of mosquito control, information and education campaigns, epidemiological and entomological surveillance. The Brazilian government had invested significantly in anti-*Aedes* campaigns, but these campaigns failed to halt the spread of dengue fever and other diseases.

Methodology: Alternative techniques and methods are under evaluation in Brazil, in order to expand the current control methods established by the PNCD. The project *Eliminate Dengue*, using symbionts (*Wolbachia*) to promote vector population suppression and block disease transmission is currently being tested and demonstrating promising results. The Sterile Insect Technique (SIT), releasing irradiated sterile males, as well as the combined SIT / IIT (*Wolbachia*-based Incompatible Insect Technique) will also be tested in the country for the suppression of *Aedes aegypti* populations. A transgenic-based approach using RIDL (Release of Insects carrying a Dominant Lethal gene) strain of *Aedes aegypti* has also been developed for population suppression. Collaboration between Moscamed, University of São Paulo and Oxitec Ltd. was established to evaluate RIDL technology in Brazil. Regulatory approval was obtained from CTNBio for general operational activities including field releases for mosquito population suppression.

Results: The *Projeto Aedes Transgenico* (PAT) covered different aspects, including mosquito mass-rearing, field releases, *Aedes* population monitoring and community awareness. PAT took place in two areas between 2010 and 2016. During the first year, the technology was adapted to local optimization of scalable mass-rearing methods. In order to be able to release efficient males in the most distant area, a pupae-transport system was also successfully developed. All in all, we were able to demonstrate mating compatibility between wild-type population and the RIDL transgenic line, as well as a male dispersal up to 200 m, with a recapture rate of 3.2 days.

Around 18 million males released in Juazeiro city were able to suppress *Ae. aegypti* population by around 60-70% (ovitrap index and number of eggs/trap). In Jacobina approximately 49 million mosquitoes were released with a population reduction of 72%.

Conclusion: The project has provided a substantially better understanding of the release of genetically modified mosquitoes (GMM), protocol for mass-rearing and community awareness, and also provided stimulus to develop new GMM strains which are already under construction and/or initial laboratory level evaluation, in order to determine their fitness and application.

In summary, all these technological development for vector control aim to increase the number of available tools to control more efficiently the mosquito population avoiding selection of resistant populations and maintaining populations below the disease transmission threshold.

Abstract ID 85

Novel Genetic Methods for the Area-wide Control of Insects: Gene Drive and Sex Ratio Distortion

Nikolai WINDBICHLER

Imperial College London, London, UK, Email: *nikolai.windbichler@imperial.ac.uk*

Genetic control aims to reduce the ability of insect pest populations to cause harm via the release of modified insects. One strategy is to bias the reproductive sex ratio towards males so that a population decreases in size or is eliminated altogether due to a lack of females.

We have developed a CRISPR-Cas9 sex distortion system that targets ribosomal sequences restricted to the member species of the *Anopheles gambiae* complex. Expression of Cas9 during spermatogenesis resulted in RNA-guided shredding of the X-chromosome during male meiosis and produced extreme male bias among progeny in the absence of any significant reduction in fertility.

The flexibility of CRISPR-Cas9, combined with the availability of genomic data for a range of insects, renders this strategy broadly applicable for the species-specific control of any pest or vector species with an XY sex-determination system by targeting sequences exclusive to the female sex chromosome.

An alternative strategy is the use of gene drive systems that enable super-Mendelian inheritance of a transgene and have the potential to modify insect populations over a timeframe of a few years.

We have developed CRISPR-Cas9 endonuclease constructs that function as gene drive systems in *Anopheles gambiae*, the main vector for malaria. A CRISPR-Cas9 gene drive constructs designed to target the mosquito target gene AGAP007280, resulting in a recessive female-sterility phenotype upon disruption, showed transmission rates to the progeny of >90%. Cage experiments indicate that this construct meets the minimum requirement for a gene drive targeting female reproduction in an insect population.

These findings could expedite the development of gene drives to suppress mosquito populations to levels that do not support malaria transmission.

Abstract ID 283

The Eliminate Dengue Programme - Use of *Wolbachia* to Control the Transmission of *Aedes aegypti* Associated Viruses

Peter RYAN

Eliminate Dengue Programme, Institute of Vector-borne Disease, Monash University, Melbourne, Australia, Email: *peter.ryan@eliminatedengue.com*

The *Eliminate Dengue Programme* is examining the use of inherited bacterial symbionts of insects known as *Wolbachia* as a novel method to interfere with *Aedes aegypti* associated viruses. This work has now progressed from basic bench studies into open field trials involving releases of *Wolbachia*-infected *Aedes aegypti* mosquitoes in five countries.

I will give an overview of *Wolbachia*-mosquito-pathogen interactions, including laboratory experiments that show broad *Wolbachia*-induced blocking of medically important pathogens such as dengue, Zika and chikungunya viruses, and an update on current field implementations that have resulted in the establishment of *Wolbachia* in local *Aedes aegypti* populations across a range of different ecological settings. Ongoing monitoring in these sites, for up 6 years after completion of the initial releases, indicates that *Wolbachia* is stable in the mosquito populations.

Observational data from disease surveillance programmes indicate an absence of local dengue transmission in areas where *Wolbachia* has been established, which is consistent with experimental data showing persistent *Wolbachia*-induced viral blocking in field population of mosquitoes.

Formal efficacy studies to assess the impact on disease, combined with large, city-wide implementations to refine deployment strategies, are currently underway, with the aim of developing *Wolbachia* as an effective, low cost and sustainable approach to control *Aedes aegypti* associated viruses.

Abstract ID 10

Fighting Malaria with Engineered Symbiotic Bacteria from Vector Mosquitoes

Marcelo JACOBS-LORENA

Johns Hopkins School of Public Health, Baltimore, Maryland, USA, Email: *mlorena@jhsph.edu*

Background: The unbearable burden of malaria demands urgent development of novel approaches to fight this deadly disease. Technical advances in vector biology have allowed the development of a new strategy to combat malaria, by genetically modifying the mosquito to reduce its vectorial competence. However, one crucial unresolved aspect of this approach is how to introduce transgenes into wild mosquito populations ("genetic drive").

Methodology: We are exploring an alternative approach based on the fact that the mosquito, as all higher organisms, carries microbiota in its midgut. Moreover, it is in the midgut that the most vulnerable stages of *Plasmodium* development in the mosquito take place. Rather than genetically modifying mosquitoes, our strategy is to genetically modify symbiotic bacteria for the delivery of anti-malarial effector molecules into the mosquito midgut (paratransgenesis).

Results: Previously, we have shown that the bacterium *Pantoae agglomerans*, engineered to express and secrete anti-*Plasmodium* effector molecules, strongly inhibits *Plasmodium* development in the mosquito by up to 98%. Recently, we have identified another mosquito symbiotic bacterium of the genus *Serratia* that is transmitted both vertically from female to larval progeny and horizontally from male to female during mating. These transmission properties suggest that it should be possible to introduce recombinant *Serratia* into mosquito populations in the field for the purpose of malaria control. This approach is 'low-tech' and does not involve the introduction of a new bacterium, since *Serratia* is a natural component of the microbiota of field mosquitoes.

Conclusion: This paratransgenesis approach may be "universal", as these bacteria can populate anophelines from Asia, Africa and the Americas and the effector molecules efficiently inhibit both rodent and human *Plasmodium* parasites. In addition, paratransgenesis is compatible and complementary to other tools to fight malaria, including insecticides, drugs and transgenesis.

Abstract ID 247

Paratransgenic Strategies for Arthropod Pests of Humans and Plants

Ravi DURVASULA[1], Ivy HURWITZ[1] and Arinder ARORA[2]

[1]University of New Mexico School of Medicine, Albuquerque, New Mexico, USA, Email: *tuanravi@gmail.com*
[2]Cornell University, Ithaca, New York, USA

Background: Arthropod vector-borne diseases play a major role in both human and plant health globally. Disease-associated morbidity and mortality, coupled with billions of dollars of agricultural losses attributed to insect pests, have a crippling effect on human development. Control of vector-borne diseases has relied principally on widespread insecticide use, with attendant issues of environmental toxicity, cost, harmful effects on beneficial insects and evolution of target resistance.

Paratransgenic approaches are under development for a variety of insect pests. In these strategies, symbiotic or commensal bacteria of an arthropod are engineered to produce molecules that disrupt the life cycle of a pathogen that resides within the insect. Insects that harbour the engineered bacteria can be rendered refractory to pathogen carriage and transmission, thereby disrupting or eliminating cycles of disease.

Paratransgenic approaches have been developed to tackle transmission of the parasite, *Trypanosoma cruzi*, by triatomine bug vectors and *Plasmodium* parasites by certain mosquitoes. However, field-applicable programmes for disease control remain future prospects.

Phlebotomine sand flies play a significant role globally as vectors of Leishmania parasites that cause human and animal disease on all continents. Epidemics of cutaneous leishmaniasis and related diseases are at historic proportions. Likewise, the plant pests referred to as sharpshooters (Cicadellidae) have decimated many important agricultural crops, such as grape plants in California, olive trees in Southern Europe and citrus trees in Brazil. Paratransgenic approaches are under development to address diseases transmitted by Old World sand flies and the Glassy Winged Sharpshooter. Efforts to characterize and engineer bacterial commensals of these arthropods, express heterologous molecules and establish pathogen-free paratransgenic insects will be presented.

Methodology: The sand flies, *Phlebotomus papatasi* and *Phlebotomus argentipes* have been studied from leishmania-endemic regions of Tunisia, Iran and India. Aerobic bacterial cultures have been established from gut contents of field-caught flies to identify suitable commensal bacteria for genetic manipulation. Expression plasmids for Bacillus and non-pathogenic Enterobacter isolates have been developed and expression of both fluorescent marker proteins and anti-leishmanial peptides and antibodies has been demonstrated.

In the glassy winged sharpshooter, *Homalodisca vitripennis*, the commensal bacterium, *Pantoea agglomerans* has been isolated and transformed to express several antimicrobial peptides that are toxic to the pathogenic bacterium, *Xylella fastidiosa*. Methods of transformation using a hemolysin secretion system will be presented.

Results: Paratransgenic phlebotomine sand flies and glassy winged sharpshooters have been established under laboratory settings. In *P. argentipes* and *P. papatasi*, transformation of the commensals, *Bacillus subtilis* or *Enterobacter cloacae* subspecies *dissolvens* to express GFP, mellitin, human histone B and specific engineered single chain antibodies that target surface receptors of *Leishmania major* and *L. donovani* has been demonstrated.

In sharpshooters, expression of mellitin and scorpine-like molecule by engineered *P. agglomerans* has resulted in pathogen-refractory insects that are incapable of transmitting *X. fastidiosa* to target grape plants under greenhouse settings. Detailed results will be presented.

Conclusion: Paratransgenic approaches hold promise for control of several human diseases, including leishmaniasis. We report applications of this technology to the agricultural pest, *H. vitripennis*, as well.

Abstract ID 277

RNAi Strategies in Support of Mosquito SIT Applications

Steve WHYARD, D. GIESBRECHT, D. BOGUSKI, R. ABBASI, C. CRUZ, Aditi SINGH, C. ERDELYAN and A. TAYLER

Department of Biological Sciences, Winnipeg, Manitoba, Canada, Email: *Steve.Whyard@umanitoba.ca*

The Sterile Insect Technique (SIT) is a biological control method that can reduce pest insect populations by releasing a large number of sterile males to compete with wild males for female mates to reduce the progeny produced. Typically, males are sterilized using radiation, but such methods can reduce their mating competitiveness. The method is most effective if only males are produced, but this requires the development of effective sex-sorting methods.

We are developing non-transgenic, non-radiation methods of generating fit sterile *Aedes aegypti* male mosquitoes that efficiently mate with females, preventing the production of offspring. Larval mosquitoes were fed double-stranded RNAs (dsRNAs) that induced an RNA interference (RNAi) mediated knockdown of both male fertility genes and a female-specific gene to generate a male-biased sterile adult population, which overcomes the need to sex-sort insects before release.

Variability in the efficacy of RNAi in mosquito strains has been observed, and hence, we are developing dsRNA micro-carriers that enhance the uptake and systemic spread of dsRNA within mosquitoes, to maximize the efficacy of the RNAi in mosquitoes and other insects amenable to RNAi-mediated SIT.

The sequence-specific gene-silencing mechanism of this RNAi technology renders it adaptable for species-specific application across numerous insect species. If consistent and potent male sterility and sex-sorting can be achieved using this technology, we envisage its use for traditional large-scale reared releases of mosquitoes and other pest insects.

Abstract ID 285

Efficient Sex Separation in *Aedes* Mosquitoes Using Image Analysis and Elimination of Females by Laser Beams

Carlos TUR

Empresa de Transformación Agraria, SA (TRAGSA), Valencia, Spain, Email: *ctur@tragsa.es*

The Sterile Insect Technique is an increasingly important component of area-wide integrated vector management programmes for key agricultural insect pests, and also has potential for integration into the control of mosquito populations. However, a critical bottleneck has been the lack of a system that efficiently separates them by sex, since the females must be eliminated given that they are the vehicles for transmitting diseases to humans through their bites. Nowadays, there are only artesanal mechanical methods of separation based on size dimorphism between male and female *Aedes* pupae that require much labour and increased efficiency to be able to reach the operational level at a large scale.

A new system based on image analysis capable of separating the mosquito pupae by sex and killing the females using a laser beam device has been developed. After identifying and measuring individual pupae in a continuous flow of pupae of both sexes by artificial vision software, an algorithm decides the sex of the pupae based on the information of the previously analysed pupae (self-learning) in the same batch. Then, a laser beam is directed to the females to kill them. Only the male *Aedes* mosquitoes survive and after their irradiation they can be released into the target areas.

A prototype able to process a million male pupae per day has been developed, although the capacity can be increased if needed by adding a second set of cameras and laser beam in parallel rows. The preliminary results are very promising since 99.7% of females are removed and the recovery of males of *Aedes albopictus* can reach up to 80%. These results are very satisfactory compared with those obtained by the current mechanical systems. Moreover the device is completely automatic, reducing the variability and the cost of the process considerably.

The prototype has been developed by TRAGSA, a Spanish decentralized governmental institution, under the frame of the FAO/IAEA Coordinated Research Project (CRP) “Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes”. In collaboration with the FAO/IAEA and other CRP partners, the system is being further refined and will be tested for other *Aedes* species like *Ae. aegypti* and *Ae. polynisiensis* in order to be deployed in open field pilot trials.

Session 3: Animal Health

Abstract ID 67

The Eradication of the Tsetse Fly *Glossina palpalis gambiensis* from the Niayes of Senegal Using an Area-wide Integrated Pest Management Approach that Includes the Release of Sterile Males

Baba SALL[1], Momar Talla SECK[2], Jérémy BOUYER[4] and Marc J.B. VREYSEN[4]

[1]Ministere de l'Elevage et des Productions animales/Direction des Services Vétérinaires, Dakar, Senegal, Email: *babasall@hotmail.com*
[2]Institut Sénégalais de Recherches Agricoles, Dakar, Senegal
[3]French Agricultural Research Centre for International Development (CIRAD), Montpellier, France
[4]Insect Pest Control Unit, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria

The "*Projet de lutte contre la mouche tsé-tsé et la trypanosomose dans les Niayes*" targets a total area of 7,350 km^2, of which 1,375 km^2 were infested with *Glossina palpalis gambiensis* (Diptera: Glossinidae) which were solely responsible for the cyclical transmission of trypanosomes, the causative agents of African animal trypanosomosis (AAT). The tsetse population in the Niayes was completely isolated from the main tsetse belt in the southeastern part of the country, which prompted the Government of Senegal to select an eradication strategy of the vector and the disease, as the risk of re-invasion would be minimal or non-existing.

The eradication project was implemented in different phases, i.e. following a phased conditional approach, which consisted of (1) training and commitment of all stakeholders, (2) baseline data collection and feasibility studies, (3) pre-operational activities, and (4) operational activities. During the baseline data collection and feasibility phase, entomological, parasitological, environmental and socio economic data were collected to assess tsetse distribution, population dynamics, trypanosomosis prevalence and distribution, potential environmental impact, and anticipated benefit-cost ratios.

Activities during the pre-operational phase included the establishment of a colony of tsetse originating from the target area in Senegal, competiveness studies between the sterile flies and those from the target area, development of transport methods for long-distance shipments of sterile male pupae, competitiveness of the sterile male flies after release in the target area, development of aerial release methods, including a new chilled adult release system, and development of a Maxent-based distribution model to guide the suppression, sterile male releases and the monitoring of the campaign.

During the operational phase, the fly populations were suppressed using insecticide-impregnated traps (~3000), insecticide-treated cattle (~10,000 heads) and insecticide-impregnated screens around pig pens (~300 m). This was followed by the release from the ground or by air of so far more than 2,500,000 sterile male tsetse flies.

An extensive entomological monitoring system implemented in the 3 operational blocks indicated the following: no wild fly catches since August 2012 in block 1, while in block 2 and 3 the densities of the wild tsetse populations have been reduced by > 95% at the time of writing.

Monitoring data of sentinel herds located inside and outside the tsetse-infested area indicated already a significant reduction of the AAT prevalence. The environmental monitoring demonstrated a very low impact of the project on non-target species, and the socio-economic study showed a high cost-effectiveness of the project.

The implementation approach of this project could serve as a model for future potential tsetse eradication campaigns in sub-Sahara Africa.

Abstract ID 121

Achievements and Challenges of Tsetse and Trypanosomosis Control Operations in the Southern Rift Valley of Ethiopia

Zerihun Woldesenbet GELETA, Dagnachew Beyene DEBELA and Firew Lejebo LEKA

National Institute for the Control & Eradication of Tsetse & Trypanosomosis (NICETT) of Ethiopia, Addis Ababa, Ethiopia, Email: *zerie2006@yahoo.com*

Background: The southern Rift Valley of Ethiopia is a very fertile land and has a huge potential for livestock and crop production. It is infested with a species of tsetse fly, *Glossina pallidipes*, which is an important vector of African animal trypanosomosis. The economic loss due to the disease is very large due to the diverse effects on livestock production and productivity. For the last many years efforts were made to control the disease mainly by using trypanocidal drugs for the treatment of sick animals. But due to the development of resistance to the commonly used drugs this control method was no more effective.

Methodology: A project was launched to control tsetse and trypanosomosis in an area of 25,000 km^2 in the southern Rift Valley. For population suppression of tsetse flies many techniques have been used most of which are insecticide-based. The techniques mainly used are insecticide-impregnated targets, traps, insecticide treatment of cattle (pour-on), ground spraying of tsetse resting sites and sequential aerial spray of non-residual insecticide aerosols (SAT). The techniques were used in an integrated way considering the conditions on the ground.

Results: The project has tremendous achievements and has brought major benefits to the local communities through significantly improved livestock production and productivity. Because of the tsetse suppression, the apparent density of the flies decreased by up to 95% and that of the disease by up to 90%. In addition to this the livestock population in the area has increased by more than 100% during the last 15 years. In addition, equine species which were unable to survive in the area are now the significant part of the livelihoods of the communities and have become a source of income for the communities in the area.

Pocket areas of tsetse found in remote inaccessible areas and national parks are major problems that are sources of reinvasion. The biggest challenge in these and other areas, where tsetse and trypanosomosis have been reduced, is the sustainability of the results achieved.

Conclusion: The integrated approach of tsetse and trypanosomosis control methods is effective to reduce the tsetse and trypanosomosis challenge and thereby increase livestock production and productivity. To sustain the benefits obtained from the control operations, the hot spot areas should be addressed by any available means and the communities should take over the ownership of the tsetse and trypanosomosis control.

Abstract ID 90

Mapping Landscape Friction to Locate Isolated Tsetse Populations that are Candidates for Elimination

Jérémy BOUYER[1], Renaud LANCELOT[2], Ahmadou DICKO[2], Thierry DE MEEUS[3], Marc J.B. VREYSEN[1], Philippe SOLANO[3], Laure GUERRINI[2], Ravel SOPHIE[3] and Giuliano CECCHI[4]

[1]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria
[2]French Agricultural Research Centre for International Development (CIRAD), Montpellier, France
[3]Institut de Recherche pour le Développement (IRD)
[4]Food and Agriculture Organization of the United Nations (FAO), Sub-regional Office for Eastern Africa, Addis Ababa, Ethiopia

Tsetse flies are the cyclical vectors of deadly human and animal trypanosomes in sub-Saharan Africa. Tsetse control is a key component for the integrated management of both plagues, but local eradication successes have been limited to less than 2% of the infested area. This is attributed to either resurgence of residual populations that were omitted from the eradication campaign or reinvasion from neighbouring infested areas.

In this paper, we focused on *Glossina palpalis gambiensis*, a riverine tsetse species representing the main vector of trypanosomoses in West Africa. We mapped landscape resistance to tsetse genetic flow, hereafter referred to as friction, to identify natural barriers that isolate tsetse populations. For this purpose, we fitted a statistical model of the genetic distance between 37 tsetse populations sampled in the region, using a set of remotely sensed environmental data as predictors.

The least-cost path between these populations was then estimated using the predicted friction map. This method enabled us to avoid the subjectivity inherent in the expert-based weighting of environmental parameters. Finally, we identified potentially isolated clusters of *G. p. gambiensis* habitat based on a species distribution model and ranked them according to their predicted genetic distance to the main tsetse population.

The methodology presented here will inform the choice on the most appropriate intervention strategies to be implemented against tsetse populations in different parts of Africa. It can also be used to control other pests and to support conservation of endangered species.

Abstract ID 64

Titre and Tissue Dynamics of Tsetse Symbionts in *Glossina* Interspecies Hybrids: Biological and Applied Aspects

Wolfgang MILLER and Daniela SCHNEIDER

Medical University of Vienna, Vienna, Austria, Email: *wolfgang.miller@meduniwien.ac.at*

In accordance with the definition of the biological species concept by Dobzhansky and Mayr, F_1 hybrids between different *Glossina* species suffer from high embryonic lethality, plus complete hybrid male sterility. Such hybrid sterility has drawn attention earlier when artificially produced hybrids were considered for application strategies in order to control and suppress tsetse populations in the field. Moreover, mass-release of sterile hybrids would also allow for using significantly lower gamma-irradiation dosages to sterilize wrongly sexed females than the currently applied high dosages for standard SIT ensuring complete sterilization of colony-bred males.

Recent studies, however, strongly suggest that such rigorous irradiation treatments of male insects for standard SIT not only damage host spermatogenesis as desired, but also harm dividing somatic cells of gut epithelia, organelles, gut microbiota, as well as their native endosymbionts. Therefore the potential mass-generation of naturally sterile *Glossina* hybrid males would improve significantly the fitness of released males in nature to fight vector-borne diseases such as trypanosomiasis.

As recently demonstrated, symbiotic microbes can affect insect host fitness and fecundity, pathogen protection as well as their mating competence and success. Hence, rigorous sterilization of tsetse males with high dosages of gamma-irradiation could also destabilize or even demolish the complex symbiotic interactions between tsetse host and the native symbionts *Wigglesworthia*, *Sodalis* and *Wolbachia*. The tempting concept of applying hybrid-males that express innate sterility, however, was hindered by the fact that the potential large-scale generation of F_1 hybrids for mass-production and release is extremely laborious and hence not feasible. Only the establishment of stable hybrid colonies, i.e., the creation of a species nova tsetse fly, being incompatible per se with native *Glossina* species at their release site would circumvent this important biological and technical issue for future, successful pest control management. Such artificially generated host hybrid backgrounds, however, might have a massive impact on native symbiont loads.

As recently reported by our group, interspecies hybrids of neotropical *Drosophila* species dramatically increase titres of their native, mutualistic *Wolbachia*. Furthermore we demonstrated that loss of symbiont titre control in hybrids triggers interspecies incompatibilities, such as high embryonic F_1 mortality and complete male sterility. Upon *Wolbachia* knockdown in parents before mating, however, hybrid sterility can be partially rescued giving rise to fertile F_1.

Most similar to this system, we recently found that in the *Glossina morsitans* species complex, interspecies hybrids also exhibit increased *Wolbachia* loads compared to their corresponding non-hybrid parents. In addition, we have uncovered accompanying mild titre alterations in *Sodalis* and *Wigglesworthia* upon hybrid formation. However, not only general symbiont load, but also spatial distribution of symbionts might be altered in artificial hybrid backgrounds. As demonstrated in weevils, loss of host control over native symbiont localization has detrimental effects as it results in spreading of the primary symbiont in previously uninfected host tissues.

Here we report on analysis of the spatial distribution of *Glossina* symbionts in artificial hybrid backgrounds, which is of pivotal interest for any further application in pest control management.

Abstract ID 91

Paratransgenesis as a Tool to Block Trypanosome Transmission by Tsetse

Jan VAN DEN ABBEELE

Institute of Tropical Medicine Antwerp, Antwerp, Belgium, Email: *jvdabbeele@itg.be*

Background: African trypanosomiases are parasitic diseases of medical and veterinary importance affecting sub-Saharan Africa. The causative agents, the African trypanosomes, are blood-borne protozoans that are transmitted through the bite of tsetse fly species (*Glossina* spp.). While human African trypanosomiasis has reached the point where eradication seems to be achievable, animal African trypanosomiasis, mainly caused by *Trypanosoma congolense* and *T. vivax*, remains one of the most important livestock diseases, particularly affecting cattle and representing a major obstacle in the agricultural economics of sub-Saharan areas.

Control of tsetse-transmitted trypanosomiases is based upon two main pillars: drug treatment of infection-diagnosed hosts and suppression and/or eradication of the tsetse populations. This vector control relies on the use of a variety of complementary techniques and combinations: insecticide-treated targets and/or animals, area-wide insecticide applications, and the Sterile Insect Technique (SIT).

A potential novel technology that is being explored in combination with the SIT, involves rendering tsetse flies refractory to trypanosome infection via the introduction of genetically modified symbiotic bacteria called paratransgenesis. Here, the tsetse's commensal bacterium, *Sodalis glossinidius*, is an ideal candidate as a bacterial paratransgenic platform. It is maternally transmitted to developing intrauterine offspring and lives in close proximity to the pathogenic trypanosomes especially in the tsetse midgut.

Methodology: We have delivered the experimental proof-of-concept that the *Sodalis* commensal can be genetically engineered to express and release functional anti-trypanosome nanobodies in *in vitro* culture conditions, as well as *in vivo* in the tsetse fly, including the midgut. Moreover, we successfully developed a Tn7-based methodology for an efficient chromosomal transgene insertion in *Sodalis*, allowing stable and strong expression of different selected nanobodies that target the tsetse procyclic midgut trypanosomes.

Results: We succeeded in a sustainable colonization of the adult tsetse flies and their subsequent offspring with genetically modified *Sodalis* by means of microinjection of newborn third instar larvae. This implies that a tsetse fly colony can be established with flies harbouring genetically transformed *Sodalis* which is an essential requirement for the massive release of paratransgenic trypanosome-resistant tsetse flies in the context of SIT.

We have demonstrated that, beside maternal transmission, the commensal bacterium can also be paternally transmitted to the offspring through the male-to-female delivery of a *Sodalis*-

containing spermatophore. This paternal transfer could provide an additional route for introducing the genetically engineered commensal into the natural tsetse fly population.

Conclusion: The methodologies to establish a paratransgenic tsetse fly colony have been developed. However, a last but difficult hurdle for rendering paratransgenic tsetse flies refractory to trypanosome infection remains the identification of an expressible component that is active in the tsetse midgut and with a high trypanolytic potency, blocking parasite transmission by the tsetse fly.

Abstract ID 252

Genetic Structure, Demographic and Phylogeographic History of the New World Screwworm Fly in South America and Caribbean Regions

Ana Maria AZEREDO-ESPIN[1,2], Luana BERGAMO[1,2], Pablo FRESIA[3], Robin MADUEÑO[4], Thiago Mastrangelo[1,2] and Mariana Lyra[5]

[1]Departamento de Genética, Evolução e Bioagentes (DGEB), Instituto de Biologia, UNICAMP, São Paulo, Brazil, Email: *amlazeredo@gmail.com*
[2]Centro de Biologia Molecular e Engenharia Genética-CBMEG, UNICAMP, São Paulo, Brazil
[3]Instituto Pasteur, Montevideo, Uruguay
[4]Dirección de Sanidad Animal, SENASA, Peru
[5]Departamento de Zoologia, Instituto de Biociências, UNESP, São Paulo, Brazil

Cochliomyia hominivorax Coquerel, 1858 (Diptera: Calliphoridae), the New World screwworm fly (NWS), is an endemic fly of the Americas. The larval stages of this insect feed on living tissue of warm-blooded hosts, including humans. Larval infestation causes serious tissue injuries and can lead to host death if not treated. For that reason, *C. hominivorax* is considered an important livestock pest that causes substantial profit losses to livestock breeders.

The current geographic distribution of NWS includes South America and some Caribbean islands; it was eradicated from North and continental Central America between 1957 and 2000 after the area-wide implementation of the Sterile Insect Technique (SIT). Planning a similar area-wide control programme in South America could be an interesting alternative to the exclusive use of insecticides, but requires the delineation of regions and geographic scales.

Studies conducting in our laboratory that investigated the genetic structure of NWS fly populations and dispersal corridors have indicated that the distribution pattern of the species is complex. Based on this scenario, the present work focused on the mode and time of divergence of the regional groups of *C. hominivorax* on the continental scale. We used mitochondrial DNA sequences to generate phylogenetic reconstructions and habitat suitability models to construct alternative hypothesis of population divergence. An approximate Bayesian computation (ABC) approach was used to test these competing demographic history scenarios for *C. hominivorax* to identify contemporary and historical events that shaped the current population structure of this insect pest.

We analyzed three mitochondrial DNA regions; the gene Cytochrome c oxidase subunit I (COI); gene Cytochrome c oxidase subunit II (COII) and control region (CR) of 550 *C. hominivorax* individuals from 75 locations in South, Central and North America. In addition to these samples, we sequenced two individuals of *Cochliomyia macellaria* Fabricius 1775,

one of the other three species of the genus, to be used as out-group in the phylogenetic analysis.

The association of this species with vertebrate hosts and its status as an insect pest suggest that the genetic and geographic distribution of this species is influenced by human activities. The genetic structure of NWS populations has already been analysed on different geographical scales. The phylogeographic studies of *C. hominivorax* across its distribution revealed the continental-scale geographic structure of the populations and identify four regional groups (Cuba, Dominican Republic, and North and South Amazon regions). At intra-group level, the authors did not find that the population structure was associated with geography. The results suggested that the human and livestock mobility in the continent during the last ~500 years might have obscured the historical phylogeographic pattern of NWS on this smaller scale, but not on the continental scale.

The ABC analysis supports that populations spread from North to South in the Americas, in at least two different moments. The first split occurred between the North/Central American and South American populations at the end of the Last Glacial Maximum (15,300- 19,000 YBP). The second split occurred between the North and South Amazonian populations in the transition between the Pleistocene and the Holocene eras (9,100- 11,000 YBP). The species also experienced population expansion.

The phylogeographic patterns observed in *C. hominivorax* cannot be explained only by climatic oscillations and can be connected to host population histories and these patterns are very coincident with general patterns of ancient human movements in the Americas, suggesting that human might have played a crucial role in shaping the distribution and population structure of this insect pest. The genetic diversity and population structure across the Brazilian Amazon region (Amazonia) data supported the existence of much diversity and significant population structure among nine regional NWS populations, which were found common in Amazonia.

The investigation of genetic variability and structure of NWS samples from different Peru regions, an unexplored country from South America that seems to be an important region for the dispersion of the species and connection between distinct areas, is being conducted in our laboratory. The preliminary results suggest the presence of four genetically distinct groups with some geographic restriction. The ten sampling locations analysed presented high haplotype diversity, low nucleotide diversity and Tajima's D and Fu's FS significant negative values, indicating a probable demographic process of population expansion.

The obtained results point out the necessity of understanding population structure and demographic dynamics on a finer spatial scale. The NWS genetic diversity distribution pattern analysed represents a critical step toward the management unit identification of this pest and is necessary to develop a more effective and preventive pest management strategy for the Caribbean and South America.

(Financial support: FAPESP, CNPq).

Abstract ID 113

Area-Wide Management of Stable Flies

David TAYLOR

USDA-Agricultural Research Service (USDA-ARS), Agroecosystems Management Research Unit, Lincoln, Nebraska, USA, Email: *dave.taylor@ars.usda.gov*

Stable flies (Diptera: Muscidae) are among the most damaging pests of livestock worldwide. Their painful bites cause both physiological and behavioural changes that reduce productivity and well-being of domestic animals and humans alike. Immature stable flies develop in decomposing and fermenting vegetative materials, often mixed with manure and other animal wastes.

Historically, stable flies have been considered to be primarily pests of confined animals and associated barnyards. Recent changes in animal husbandry and agronomy, combined with the broad adaptability of stable flies, have exasperated the problem by producing substrates conducive for their development in pastures and croplands. Serious outbreaks of stable flies attributed to crop residues have been reported in several countries including Australia, Brazil and Costa Rica.

Infestation levels in excess of 1,500-2,000 flies per animal have been reported. Such infestations can reduce animal productivity to near zero and produce mortality. These outbreaks have also produced social unrest as ranchers, dairymen and subsistence livestock producers face the inability to produce a profitable product or, in some cases, even maintain viable herds because of stable fly infestations associated with new crops.

Area-wide and integrated management techniques are required to address this issue because of the broad geographical scale, environmentally sensitive lands and dispersal capacity of stable flies. Inadequate control is currently being achieved by incorporating cultural modifications to reduce developmental substrate suitability and treatment with Insect Growth Regulators in conjunction with adult trapping, on-animal repellents and premise sprays.

Improvements in cultural practices and traps, additional products for treatment of substrates to reduce potential development of resistance, and biological control options are needed to maintain stable fly populations below economic threshold levels. Because both male and female stable flies feed on blood, effective gene drive mechanisms must be developed before genetic control options can be viable.

Session 4: Regulatory Issues and Socio-Economic Impact

Abstract ID 148

Will the "Nagoya Protocol on Access and Benefit Sharing" Put an End to Biological Control?

Joop VAN LENTEREN

Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands, Email: *joop.vanlenteren@wur.nl*

As a result of the 1992 Convention on Biological Diversity (CBD) three objectives were formulated: (a) conservation of biological diversity, (b) sustainable use of its components and (c) the fair and equitable sharing of benefits arising out of the utilization of genetic resources. The CBD is an international framework convention, and its provisions are binding on its contracting parties, but the CBD is unable to prescribe how decisions are to be implemented by the parties since different countries have different legal structures.

It is now internationally recognised that countries have sovereign rights over the biological resources within their boundaries and so agreements governing the access to these resources and the sharing of the benefits arising from them should be established and agreed between the parties involved. To facilitate the implementation of this principle, in 2002 the Conference of Parties (COP) to the CBD adopted the 'Bonn Guidelines on Access to Genetic Resources and Fair and Equitable Sharing of the Benefits Arising out of their Utilization' (SCBD 2002, Decision VI/24).

Genetic resources are defined by the CBD as genetic material, i.e. material containing functional units of heredity that is of actual or potential value (CBD Art. 2), which includes all biological control agents. The value of the genetic resources need not be commercial (i.e. monetary), but may be scientific or academic in nature. As the CBD definition also includes the potential value of such resources, in effect all genetic material falls under the provisions of the *Access and Benefit Sharing* (ABS) system. In 2010 the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation was accepted as a multilateral legal instrument within the CBD. It has now come into force, having been signed by 92 and ratified by 68 countries.

Many biological control practitioners are still unaware of the implications of ABS under the CBD. Biological control (BC) – the use of an organism to reduce the population density of another organism – is one of the most environmentally safe and economically profitable pest management methods. In BC, parasitoids, predators, pathogens, herbivores and antagonists are used to reduce populations of pests, diseases and weeds.

Alien species are being introduced accidentally around the world at an increasing rate, driven by factors such as growing trade, travel and tourism, and such introductions have resulted in the establishment many exotic pests, diseases and weeds. In contrast, deliberate introductions and releases of exotic BC agents have resulted in remarkably few problems, and usually a

risk analysis for non-target species is now performed for BC programmes prior to introduction and release.

BC does not fall under *bioprospecting* (the search for plant and animal species from which medicinal drugs and other commercially valuable compounds can be obtained) or *biopiracy* (bioprospecting without permission of the country that owns the genetic resources and which exploits plant and animal species by claiming patents to restrict their general use). Bioprospecting or biopiracy are often concerned with products that can be protected with intellectual property rights in order to generate monetary profits for companies (e.g. pharmaceuticals and seeds).

ABS is a potentially serious threat to the use of BC because of the necessity of agreements governing access to genetic resources and sharing of benefits arising from their use between the parties involved. ABS applies to all BC agents taken from one country to another. BC practitioners will need to comply with whatever ABS regime is agreed by the Conference of the Parties to the CBD. Recent applications of CBD principles have already created barriers to collection and export of natural enemies for BC research in several countries. If this approach is widely applied it will seriously interfere with this very successful and environmentally safe pest management method.

During my presentation I will describe the current state of affairs with regard to ABS and also explain actions undertaken by the International Organization for Biological Control (IOBC) together with the Centre for Agriculture and Biosciences International (CABI) to develop fine-tuned and workable protocols for collection and exchange of organisms for scientific research and non-commercial biological control programmes.

Abstract ID 145

The Relationship between the Phytosanitary Regulatory Framework and International Trade – Case Studies

Stephanie BLOEM

North American Plant Protection Organization (NAPPO), North Carolina, USA, Email: *stephanie.bloem@nappo.org*

In its broadest sense, a regulatory framework brings together legislation and other important statutes that support the direction or implementation of a proposed course of action. In the case of plant health, this framework includes national regulations, positions and actions, as well as regional and international standards that allow countries to work towards protecting the plant resources of their country.

Apparent contradictory interests between international trade, which has facilitated the spread of plant pests, and the protection of plants, are mutually recognized in global trade and phytosanitary agreements. Each country has a National Plant Protection Organization (NPPO) responsible for developing, adopting and enforcing their national plant health legislation and regulations.

Regional Plant Protection Organizations (RPPOs) contribute to the regulatory framework by focusing on regional plant health issues and developing tools to foster cooperation in plant health at this level. Internationally, the International Plant Protection Convention (IPPC) aims to secure coordinated, effective action to prevent the introduction and spread of pests of plants and plant products.

RPPOs and the IPPC develop and adopt standards for phytosanitary measures that assist the member countries and contracting parties in protecting plant resources. The IPPC has always played an important role in international trade. The relationship of the IPPC to international trade is strengthened by the World Trade Organization's Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), which names the IPPC as the international organization responsible for phytosanitary standard-setting and the harmonization of phytosanitary measures affecting trade.

Through the discussion of several relevant case-studies, this presentation will focus on how regulatory actions and regional and international plant health standards have contributed to successful outcomes when attempting to deal with a number of high impact pests that are associated with and adversely impact international trade.

Abstract ID 263

Regulatory and Societal Considerations of New Genetic Techniques

Kaare Magne NIELSEN[1,2]

[1]Department of Life Sciences and Health, Oslo and Akershus University College, Oslo, Norway
Email: *kaare.nielsen@hioa.no*
[2]Genøk-Center for Biosafety, Tromsø, Norway

Site directed nuclease technology offers new opportunities for targeted genome modifications. A key question is if new gene modification processes / products are sufficiently consistent as a group, and distinguishable from current GMOs that can warrant a different regulatory status. This in contrast to current GMOs that are regulated as a single group: implying consistent characteristics and a similar risk profile. Regulation in the EU is process triggered and also the new techniques make use of technology developed for genetic modification purposes.

Here I explore to what extent new techniques warrant new regulatory approaches and their broader societal context.

Abstract ID 66

Avoiding Trade Restrictions due to Questionable Assertions about Taxonomy, Hosts, and Geographic Distribution of Regulated Pests

Guy HALLMAN

Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria, Email: *g.j.hallman@iaea.org*

Trade in agricultural commodities is highly susceptible to assertions about regulated pests. For example, when new economic species of the genus *Bactrocera* (Diptera: Tephritidae) were named over 20 years ago new trade barriers were erected because phytosanitary measures to prevent the introduction and spread of these new species did not exist. The lack of voucher specimens from previous research into phytosanitary measures hampered the ability to retroactively apply these measures to the new species. By the time it became widely accepted, 20 years later, that some of the newly named species were not valid the economic, damage had been suffered.

Contrastingly, the existence of multiple species among populations considered as *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) is scientifically defensible as it is based on exhaustive, multi-faceted studies. The imminent description of new species in this case may also lead to new trade barriers. Again, voucher specimens could have helped avoid some of this potential disruption.

Similar situations exist with host lists and geographical distributions that may or may not be scientifically defensible, and these situations are expected to increase and become more difficult to interpret as changes in climate worldwide develop. Erroneous assertions of taxonomic, host or geographic status of regulated pests do not cause direct but indirect economic damage manifested by loss of markets.

Scientists as individuals and communities can work to avoid this needless economic damage by agreeing to some basic standards when making assertions about regulated pests and resisting the urge to get novel information quickly into the literature. Guidelines are available; a primary source for relevant guidelines is the International Plant Protection Convention's International Standards for Phytosanitary Measures, which are available on-line in various languages.

Abstract ID 232

Methods to Quantify Pest Risk to Promote Objective Regulatory Decision-making: Case Studies on Sterile Insect Techniques (SIT) Related Programmes

Trang VO

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ), Maryland, USA, Email: *trang.t.vo@aphis.usda.gov*

Integrating economics in the traditional decision framework for managing invasive species enables thresholds of pest impacts to be determined consistently and rationally across pest species.

Using economic principles, decision criteria for determining public response are established to guide U.S. federal actions on new pest events. This enables regulatory actions to be based on both economic efficiency and biological impact grounds.

The application of the decision criteria is demonstrated in selected case studies including that involving the management of the pink bollworm (*Pectinophora gossypiella*) in the United States.

Abstract ID 212

Public-private Partnerships: How Much Should Producers Pay and How Much Should Governments Subsidize Area-wide Programmes

John MUMFORD

Imperial College London, Berkshire, UK, Email: *j.mumford@imperial.ac.uk*

Who pays for area-wide pest management is both a political question and a market decision. Partnerships work when they deliver mutual benefit, so the key issue in determining value to partners, both public and private, is a calculation on the net benefits each partner receives.

Partners may have a range of different interests: discoverer, investor, user, operator, or agent. The benefits to each cover different time periods, and various direct or indirect benefits. Discovers develop the intellectual property and make an investment decision on the basis of the cost of their effort and facilities against the sale or license of the intellectual property. Other investors may support capital facilities, such as sterile insect rearing facilities, in return for long-term contracts for the outputs. For users and operators, direct monetary return is the key benefit, and may be translated into an annual charge for a service that brings benefits in the same season. An agent, such as a government department, acting on behalf of a wider public interest, must consider more diverse returns that include both direct and indirect benefits.

Political considerations may affect the time horizon on such benefits. Apart from the interests of the various partner groups the purpose of the area-wide management also affects the calculation of benefits: eradication, suppression or prevention. These purposes will determine the temporal and spatial scales of the activities, any economies of scale, and the type of partner interests that are relevant. Economic decisions are often considered in relation to optimising returns, but other objectives may also be valid. For example, area-wide preventive control may be viewed as an insurance, as could support for local eradication by partners outside the eradication zone.

Much area-wide pest management has traditionally been supported by government on the basis of it being a public good. However, governments and the public they serve are increasingly expecting a degree of co-responsibility and cost-sharing by private beneficiaries. Various legal and contractual arrangements can determine the respective responsibilities of public and private partners.

Abstract ID 123

Costs and Benefits of Queensland Fruit Fly Area-wide Management with Linked Spatial Population Simulations

Tim CAPON[1], Jeff CONNOR[1], John KANDULU[1], Penny MEASHAM[1,2], Hazel PARRY[1], Florian SCHWARZMUELLER[1], Mia TAM[3], Stuart WHITTEN[1] and Nancy SCHELLHORN[1]

[1]Commonwealth Scientific and Industrial Organization (CSIRO), Canberra, Australia, Email: *tim.capon@csiro.au*
[2]Horticulture Innovation Australia (HIA)
[3]Primary Industries and Regions South Australia (PIRSA), Australia

The Queensland fruit fly (*Bactrocera tryoni*) has become an increasingly significant risk to the horticultural industry in south-eastern Australia. The Sterile Insect Technique (SIT) is being introduced as a new method of control. To be successful, however, it needs to be adopted into an Area-Wide Management (AWM) strategy, and evaluated for biophysical and economic feasibility.

Here, we report on our progress in evaluating the benefits and costs of implementing AWM with the SIT for *B. tryoni*. The approach links spatial simulation modelling of the meta-population dynamics of *B. tryoni* with economic modelling of the costs and benefits of AWM.

We present a range of control scenarios in landscapes with different spatial configurations. This analysis gives an indication of the biophysical effectiveness and potential costs and benefits of AWM, leading towards regionally-specific guidelines for implementing AWM using SIT.

Session 5: Climate Change, Global Trade and Invasive Species

Abstract ID 94

Global Trends in Arthropod Eradication Programmes

David Suckling

University of Auckland and The New Zealand Institute for Plant and Food Research, Christchurch, New Zealand, Email: *max.suckling@plantandfood.co.nz*

The *Global Eradication Database* (http://b3.net.nz/gerda) has continued to grow case studies of incursion responses and eradications related to mosquitoes, fruit flies, Lepidoptera, ants and other groups of invasive arthropods. The rate of new programmes being commenced continued to rise on a logarithmic scale during the 20th century. In the case of Lepidoptera other than gypsy moth, 75% of programmes have been started in the last 20 years, supporting a case for rapid geographic range expansion under globalisation.

For all arthropods, more than 80% of eradication programmes have been successful, although for certain groups such as fruit flies of economic importance, the success rate is even higher. Arthropod eradications often use multiple tactics, but confounding effects make it difficult to determine the effectiveness of single tactics.

A steady increase in the number of eradication programmes globally suggests that current measures for constraining the spread of invasive species are not adequate.

Abstract ID 60

Area-Wide Management of Invading Gypsy Moth (*Lymantria dispar*) Populations in the USA

Andrew LIEBHOLD and Donna LEONARD

[1]USDA Forest Service, West Virginia, USA, Email: *aliebhold@gmail.com*
[2]USDA Forest Service Forest Health Protection, USA

The problem of biological invasions has arisen as an inadvertent side-effect from globalization. While major advances have been made to implement measures to prevent future invasions, these efforts are never perfect and new, potentially damaging species continue to establish. When such new invasions occur, it is often possible to detect them early enough such that they can be eradicated or spread of the species can be contained.

Here we describe the management of invading gypsy moth, *Lymantria dispar*, populations in the USA as a model of how area-wide management can be used to manage insect invasions. This insect was accidentally introduced near Boston in 1869 and has slowly expanded its range through eastern North America. The species is currently established in ca. 1/3 of its potential North American range and during recurrent outbreaks, populations cause massive forest defoliation.

The management of gypsy moth invasion in the USA consists of two components: 1) surveillance, detection and eradication of nascent populations in currently uninfested regions and 2) suppression of invading populations along the expanding population front (containment). Both programmes utilize extensive grids of pheromone-baited traps to detect new populations; in both programmes, roughly 100,000 traps are deployed annually.

When populations are detected in one or more traps, a higher density of traps is deployed to confirm the persistence of the population and to delimit its spatial extent. Detection and delimitation is then followed with treatment. In eradication programmes, most treatments utilize aerial application of the bacterial pesticide *Bacillus thuringiensis*. Most treatments in the containment programme use mating disruption. Following treatment, grids of additional traps are deployed to confirm eradication.

Both programmes have been quite successful at slowing the rate at which this insect has invaded uninfested regions. This success can be attributed in part to the successful application of technology, including geographical information system technology which plays a central role in both programmes.

Abstract ID 80

Mediterranean Fruit Fly Eradication Programme in the Dominican Republic

Gregory MARTE[1], José Luis ZAVALA[2] and Francisco MARTÍNEZ PUJOLS[1]

[1]Ministry of Agriculture, Moscamed-RD, Santo Domingo, Dominican Republic, Email: *Gregory.marte@agricultura.gob.do*
[2]FAO/IAEA Consultant, Higuey, Dominican Republic

Background: The presence of the Mediterranean fruit fly, *Ceratitis capitata* (Wied), was officially reported in the Dominican Republic in March 2015. The pest had already spread to 2,053 km^2 in the eastern part of the Dominican Republic, constituting a mayor outbreak. An immediate ban to exports of fruit and vegetables was imposed by trading partners, causing a loss of US $40 million in a short period of time.

In response, the Ministry of Agriculture established the Mediterranean fruit fly Programme, providing the required financial and operational support. The FAO/IAEA and APHIS-USDA provided support to the country, together with assistance from regional organization such as IICA and OIRSA. The Guatemala-México-USA Moscamed Programme played a major role in assisting through technology transfer, which included application of the SIT and integrated pest management components.

Methodology: A country-wide trapping network was deployed to assess the extent and distribution of the infestation. Fruit sampling was also implemented. It was determined that the infestation was present in the Eastern Region of the country, with detections in the provinces of La Altagracia, La Romana, El Seibo, San Pedro de Macoris and Hato Mayor, and the heaviest infestation in the Punta Cana area, a touristic area with no commercial fruit tree orchards.

For population suppression, ground and aerial GF 120 bait spray was applied. A quarantine network was imposed. When the Mediterranean fruit fly infestation was very well defined and the population reduced, SIT application was started using ground releases and later aerial releases using the chilled adult system with an area-wide approach.

Results: From 5 April 2015 to 26 November 2017, 3,936 males and 235 females in 14,541 installed traps country-wide and 1,189 larvae in 7,168 fruit samples have been detected. It was established that the primary host were wild almond (*Terminalia cattapa*), caya (*Sideroxylon foetidisimum*) and guava (*Psidium guajava*). 47,956 litres of insecticide bait spray have been applied, 1190 tons of infested fruit have been destroyed, and 2,397 million of sterile flies released.

With these actions, substantial progress in Mediterranean fruit fly eradication has been achieved. Current eradication actions are being implemented in 44 km^2. Control technology and a reliable trapping network is in place for early detection. Trapping network has been

restructured based on risk factors, placing traps in ports of entry, host areas, touristic sites, markets and sites where pest presence was recurrent. The export ban was lifted in early 2016.

Conclusion: Eradication of the Mediterranean fruit fly from Dominican Republic is in progress and expected to be achieved by mid-2017. The country has developed the capacity for early detection and emergency response and for area-wide application of the SIT.

This valuable experience could be shared with Haiti and throughout the Caribbean Region to strengthen the surveillance systems and to prevent similar situations, which can result in serious economic losses with important socioeconomic impact.

Key words: Mediterranean fruit fly, eradication, SIT, IPM, area-wide

Abstract ID 112

Drosphila suzukii Invasions and Options for its Management

Hannah BURRACK[1], Joanna CHIU[2], Kent DAANE[3], Lauren DIEPENBROCK[1], Rufus ISAACS[4], Greg LOEB[5], Cesar RODRIGUEZ-SAONA[6], Ash SIAL[7], Katherine SWOBODA-BHATTARAI[1] and Vaughn WALTON[8]

[1]North Carolina State University, Raleigh, North Carolina, USA, Email: *hjburrac@ncsu.edu*
[2]University of California, Davis, California, USA
[3]University of California, Berkeley, California, USA
[4]Michigan State University, East Lansing, Michigan, USA
[5]Cornell University, Ithaca, New York, USA
[6]Rutgers University, New Brunswick, New Jersey, USAJ
[7]University of Georgia, Athens, Georgia, USA
[8]Oregon State University, Corvallis, Oregon, USA

Drosophila suzukii, referred to as the spotted wing drosophila, is a globally invasive pest of soft skinned fruit crops, including blueberries, cherries, blackberries, raspberries, strawberries, and grapes. Since its initial detections in North America and Europe in 2008, *D. suzukii* has become established in at least 22 countries beyond its presumed native range in mainland Asia. This rapid range expansion has been accompanied by significant economic losses in host crops. If left unmanaged, *D. suzukii* infestation can result in total crop loss, but even very low infestation rates are of economic concern because fresh fruit purchasers have zero tolerance for infestation. The detection of a single infested fruit can result in total crop rejection.

Short term management efforts for *D. suzukii* have been insecticide-centred because limited information about pest biology in its expanded range was available. There are limited insecticides that are effective against *D. suzukii* and, of the effective materials, fewer still which can be used during the harvest period when *D. suzukii* damage occurs. The increase in insecticide usage in response to *D. suzukii* is not economically or environmentally sustainable.

Large-scale coordinated projects have been undertaken in the United States and Europe to develop biologically-based management strategies. A broad range of tactics are under development in the United States and include population modelling, development of monitoring tools, improved insecticide application technologies, classical biological control, cultural controls, post-harvest treatments, and genetic pest management.

An integrated strategy, combining a number of different tactics, will be necessary for long-term *D. suzukii* management.

Abstract ID 132

Invasion of Europe with Vector-borne Disease Pathogen Transmitting Mosquitoes

Eva VERONESI

University of Zürich, Zürich, Switzerland, Email: *eva.veronesi@uzh.ch*

In the last decades, the whole of Europe has experienced several waves of incursion from exotic species of mosquitoes involved in the transmission of vector-borne pathogens. Among these species, *Aedes albopictus* and *Ae. aegypti* are the most competent for transmission of human pathogen such as chikungunya (CHIKV), dengue (DENV) and zika virus (ZIKV).

Other invasive exotic species such as *Ae. japonicus*, *Ae. koreicus*, *Ae. cretinus*, and *Ae. triseriatus* are also present with established or sporadic presence in many European countries. A better knowledge on the expanding distributions of these species is of paramount importance for risk assessment and management of the diseases caused by the pathogens they transmit.

This paper will give an overview on the role that exotic mosquito species present in Europe have on the transmission of pathogens. Peculiarity on their ecology, vector capacity and transmission mechanisms, together with an update of vector-borne diseases distribution in the Europe region, will also be elucidated.

Abstract ID 55

Climate and Mediterranean Fruit Fly Invasion Persistence: Insights from Agent-Based Simulations

Nicholas MANOUKIS

US Department of Agriculture, Agricultural Research Service (USDA-ARS), Hilo, Hawaii, USA, Email: *nicholas.manoukis@ars.usda.gov*

Background: Ceratitis capitata (Mediterranean fruit fly) is a major threat to agriculture around the world because it can infest a large variety of commercial fruit crops, is able to persist in a wide variety of habitats, and is known to have high invasive potential. Multiple introductions of this tephritid pest occur annually, imposing significant economic costs on government agencies and growers. Therefore, understanding when and where such introductions will be difficult to extirpate or are most likely to lead to an establishment is important.

Methodology: We used an agent-based simulation (ABS), originally developed to estimate the time to extirpation of Mediterranean fruit fly in areas where it was not established, to assess the impact of introductions across time and space. We simulated a "standard" outbreak and the usual response seen in areas where Mediterranean fruit fly is absent, including quarantines and eradication efforts via trapping, fruit removal, chemical control and the SIT. We focused especially on California and Florida (USA), and analysed differences between locations, between weeks within years, and between years.

Results: We define outbreak severity as the difficulty in eliminating an invading population of Mediterranean fruit fly. Our analysis suggests a high degree of variability in the severity of outbreaks between locations, driven by climatic differences. Cities such as Miami, Florida are too hot during the summer months to provide good conditions for Mediterranean fruit fly, while San Francisco (California) is more amenable year-round. We also found differences in outbreak severity within a single location, depending on the time of year when the introduction occurs. For example, an outbreak in Los Angeles, California in winter tends to be harder to eradicate than one that begins in summer.

We also found significant differences in the seasonal variation of outbreak severity in some locations depending on the year, which we suspect is attributable to ENSO ("El Nino Southern Oscillation") events. We present results from outbreak records to validate the observations from hundreds of thousands of ABS simulations.

Conclusion: This study indicates that programme managers and response agencies should consider long-scale climate events and predictions when designing and budgeting their eradication programmes for Mediterranean fruit fly in areas currently free of this pest.

Abstract ID 143

GIS-Based Modelling to Predict the Impact of Climate Change on Mediterranean fruit fly Populations in Central America and Decision-Making Support for Pest Management

Estuardo Enrique LIRA PRERA[1], David MIDGARDEN[2] and Pedro RENDON[3]

[1]Programa Moscamed, Guatemala-City, Guatemala, Email: *estuardo.lira@aphis.usda.gov*
[2]USDA-APHIS-International Services, USA
[3]IAEA-TCLA - Mediterranean fruit fly Programme, Guatemala-City, Guatemala

The Mediterranean fruit fly Programme in Guatemala, Mexico and Belize has implemented the use of Geographic Information Systems (GIS) since 2004. The GIS allows the integration of trapping data, control activities and environmental information which, combined with expert knowledge/interpretation (entomologist, ecologist and technical managers), provides the opportunity to conduct spatio-temporal analysis to determine the distribution of Mediterranean fruit fly populations, to recognize and understand geographic and temporal patterns, and their relationships with ecological factors and control activities.

The understanding of the relationships of ecological factors and the distribution of Mediterranean fruit fly populations allows linking the behaviour of this pest with the effects of climate change. Most of the prediction models of climate change indicate that the temperature will increase in the incoming years. Temperature is a key ecological factor for insects in general, and Mediterranean fruit fly in particular. Auclair et al. (2008) used the climate-host-insect interaction to develop predictive tools related with El Niño conditions, under the hypothesis that increase in temperatures will cause exponential Mediterranean fruit fly population growth.

The use of GIS, statistical analyses and the prediction models for climate change and El Nino conditions, allowed estimating the distribution of Mediterranean fruit fly populations under the current conditions, and with temperature increases, which are expected to be the trend as a consequence of climate change. These estimations should be considered, at least, as an early warning for pest management decisions and support the decisions to monitor and control Mediterranean fruit fly populations.

Session 6: New Development and Tools for Area-wide Integrated Pest Management

Abstract ID 16

Genome Editing and its Possible Applications: Curse or Blessing?

Marc F. SCHETELIG

Justus-Liebig-University Giessen, Germany, Email: *marc.schetelig@uni-giessen.de*

Insect vectors are responsible for carrying 20 % of all transmittable diseases affecting people in developing countries. Mosquitoes cause the biggest medical and financial burden by spreading malaria, dengue and yellow fever, Japanese encephalitis, Chikungunya and West Nile viruses. Over the last 30 years, due to increased globalization, population growth, climate change, poverty and poor sanitation, many of these diseases have been re-emerging, with the most rapidly spreading being dengue.

Chemical insecticides remain the most commonly used weapon against the vector of these diseases. Nevertheless, the establishment of resistance is undermining effectiveness of chemical insecticides for mosquito control. This creates the urgency for developing alternative strategies for vector control.

One of the most promising alternatives is the Sterile Insect Technique (SIT). Mass release of sterile male insects has proven a successful strategy for controlling many major insect pests without the disadvantages of using chemical insecticides. Important, but at the same time not available for most mosquito species, is the separation of males from females, so-called sexing system, for generating male-only populations. Here, we will present molecular systems to evaluate and compare the biological quality of insect strains and discuss options to generate sexing strains that are a key component for establishing efficient programmes for mosquito control.

Molecular systems have been integrated into several insect species by germline transformation of DNA constructs using transposable elements as a tool to randomly integrate genes of interest in a genome. While effective, in many agricultural and medical relevant species, the need of site-specific systems arose and has been answered with the establishment of several technologies that will be presented and discussed. The newest addition to these site-specific recombination technologies are the CRISPR/Cas systems that have been proven functional in many insect species and could not only be used for laboratory experiments, but also be extended for field applications.

While the use of these technologies in laboratories with adequate safety levels is an important addition for the scientific community, the use of these technologies in field applications must be thoroughly evaluated on a case-by-case study to evaluate the pros and cons and potential risks associated. We will give an overview of a few feasible scenarios and discuss contentious technical as well as regulatory issues that could help in developing an answer: are these new genome editing possibilities a curse or blessing for us?

Abstract ID 187

The Potential Addition of SIR and Bt-sugarcane to the Toolbox of an AW-IPM Programme against *Eldana saccharina* in South African Sugarcane

Desmond CONLONG and Richard RUTHERFORD

South African Sugarcane Research Institute (SASRI), Mount Edgecombe, KwaZulu-Natal, South Africa, Email: *des.conlong@sugar.org.za*

Losses to the African sugarcane borer *Eldana saccharina* are estimated to be in the region of USD 100 million per annum. The South African Sugarcane Research Institute promotes an area-wide integrated pest management (AW-IPM) approach against *E. saccharina*. This includes promoting measures that improve soil health, the use of reduced risk insecticides, a habitat management system ("push-pull") and the optimal deployment of recommended varieties on different soil types to minimise plant stress.

Transgenic crops expressing insecticidal toxins derived from *Bacillus thuringiensis* (Bt) are widely used to control insect pests. The benefits of such crops would be lost if resistance to the toxins spread to a significant proportion of the pest population. The main resistance management method, is the high-dose/refuge strategy, requiring nearby refuges of toxin-free crops, and the use of toxin doses in the transgenic crop sufficiently high to kill not only wild type insects but also insects heterozygous for a resistance allele, thereby rendering the resistance functionally recessive. Results support the idea that Bt crops are likely to be most useful when combined with other tactics. Much attention on delaying resistance to Bt crops has focused on refuge distribution, abundance and type, as well as on the number and type of toxins used in transgenic plants. However, variable farmer compliance is one of the limitations of this.

Here we propose an alternative strategy for *E. saccharina* control where sterile insects are released to mate with Bt resistant insects in the place of refuges for wild populations of the insect, or where refuges are scarce or absent. Computer simulations show that this approach works in principle against pests with recessive or dominant inheritance of resistance. During a large-scale, four-year field deployment of this strategy in Arizona, USA, resistance of pink bollworm (*Pectinophora gossypiella*) to Bt-cotton did not increase. Following the introduction of a multitactical eradication programme that included the release of sterile moths, pink bollworm abundance in commercial cotton plantations was reduced by >99%. This also eliminated the need for insecticide sprays against this key invasive pest.

Similarities in this proven approach and the planned approach to limit *E. saccharina* populations in South African sugarcane will be presented, where transgenic sugarcane and SIT are both new tools available for its integrated pest management.

Abstract ID 249

Integration of MAT and SIT

Phillip Warren TAYLOR[1] and Todd SHELLY[2]

[1]Macquarie University, Sydney, Australia, Email: *phil.taylor@mq.edu.au*
[2]USDA-APHIS, Hawaii, USA

Sterile Insect Technique (SIT) programmes rely on released sterile males securing a substantial proportion of matings with females of the targeted wild populations. Females only mated by sterile males fail to reproduce and, if enough wild females accept sterile males as mates, the wild population is diminished in subsequent generations. While sexual selection and active mate choice are common characteristics of tephritid mating systems, such that sterile males must meet the sexual criteria of females in the target population, SIT is also very much about numbers; generally, the greater the operational numerical advantage to sterile males over their fertile wild rivals, the greater the proportion of matings that will be by sterile males and the greater the efficacy of SIT. Numerous options are available for increasing pressure from sterile males on wild populations. In some species, unisex strains double the effective production of 'active ingredient' males and eliminate the sexual distraction of receptive sterile females in the field. Increased relative abundance of sterile vs. wild males can also come from increased release rates, as well as interventions such as pre-release diets and hormonal supplements that result in accelerated development or improved survival.

In many fruit fly species, the Male Annihilation Technique (MAT) is routinely used as major component of area-wide control programmes. In MAT, lures are used to attract males to toxicants. If enough males are eliminated from the wild population, a large proportion of females remain unmated and unable to reproduce. MAT and SIT are similar in that their mode of action is through increased levels of female reproductive failure; in SIT because females mate with sterile males and in MAT because females do not mate at all. Some control programmes make use of this similarity, commencing with MAT to reduce abundance of wild males and then following with SIT such that released sterile males 'replace' the eliminated fertile males as prospective mates for wild females. This approach can substantially improve the initial numerical advantages for sterile males. However, the lures used in MAT also attract released sterile males and so MAT and SIT are often envisaged as compatible sequentially, but not simultaneously. Recently, there has been increased interest in the possibility that MAT and SIT might be compatible simultaneously under some conditions, in particular if released sterile males show substantially lower responsiveness to MAT lures than do males of wild populations.

This presentation will consider historical and current practises for the sequential and simultaneous application of MAT and SIT, and the benefits and costs of each option. Interventions that show potential for reducing sterile male responses to lures used in MAT

will be explored as a largely new research frontier that might open new opportunities for substantial advances in the efficacy and cost-effectiveness of SIT.

Abstract ID 138

The Importance of Dormancy and Dormancy Managment for Biological Control

Daniel HAHN

University of Florida, USA, Email: *dahahn@ufl.edu*

The success of most biological control programmes relies on producing high-quality insects for release, from area-wide programmes with sterile insects to augmentative control with natural enemies. Here I will discuss how an understanding of insect dormancy responses may help improve biological control programmes.

Insect dormancy responses vary from programmed diapause to environmentally induced quiescence. Obligate diapause responses can be a major hurdle to mass-rearing insects for biological control programmes and I will discuss efforts to subvert obligate diapause responses in several insect pests. Similarly, mis-matching in the synchrony of seasonal dormancy responses between natural enemies and their prey can be a major hurdle for biological control programmes, and I will highlight how selection of proper diapause and overwintering responses may facilitate biological control of aquatic and terrestrial weeds.

However, dormancy and other stress responses can also be used to improve the performance of biological control agents. For example, producers may induce dormancy to increase the shelf-life of insects so that biological control agents may be held longer between production and release without loss of performance. These methods may even lead to new avenues for cryopreservation of insect stocks to maintain genetic diversity while reducing rearing costs.

For many control programmes, starting early in the spring before pest populations increase substantially is critical. However, the field environment in the early spring is often mis-matched with respect to the warm and constant environment experienced in mass-rearing facilities, with negative effects on insect performance when biological control agents could be most effective in suppressing pest populations.

Dormancy and other seasonal acclimation responses can be used to improve the performance of insects for release in the spring and fall, thereby improving the efficacy of area-wide control programmes. Similarly, a class of acclimation responses termed *hormesis*, where insects are exposed to a mild stress that boosts performance, can also be used to improve biological control agent performance, sometimes in surprising ways.

Overall, understanding and managing insect dormancy and acclimation responses offers many new avenues for improving mass-rearing, storage and shelf-life, and the field performance of biological control agents used in area-wide integrated pest control programmes.

Abstract ID 254

Characterization of Gut Microflora of Insects of Economic Importance – Practical Application

George TSIAMIS[1], Elias ASIMAKIS[1], Antonis AUGUSTINOS[1,2], Carlos CÁCERES-BARRIOS[2] and Kostas BOURTZIS[2]

[1]Department of Environmental and Natural Resources Management, University of Patras, Agrinio, Greece, Email: *gtsiamis1@gmail.com*
[2]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria

Insects are by far the most diverse and most abundant animal group with respect to the number of species globally, in ecological habitats and in biomass. The ecological and evolutionary success of insects depends on their countless relationships with beneficial microorganisms, which are known to influence all aspects of their biology, physiology, ecology and evolution.

More specifically these symbiotic associations are known to enhance: (a) nutrient-poor diets, (b) aid digestion of recalcitrant food components, (c) protect from predators, parasites, and pathogens, (d) contribute to inter- and intraspecific communication, (e) affect efficiency as disease vectors, (f) and govern mating and reproductive systems. Characterization, exploitation and management of the insect-bacterial symbiotic associations can contribute significantly in the control of agricultural pests and disease vectors.

As for essentially all animals, microbial communities are particularly prominent in the digestive tract, where they may be key mediators of the varied lifestyles of insect hosts. The contribution of microorganisms, particularly gut microorganisms, to insect function is highly relevant from several perspectives, linking to applications in medicine, agriculture, and ecology. Gut-associated bacteria can influence: (a) vectoring efficiency, (b) developmental time, (c) decomposition of plant biomass and carbon cycle, (d) nitrogen fixation and nitrogen cycle, (e) mating incompatibilities, and (f) detoxification of pesticides leading to acquisition of insecticide resistance.

For this reason, we have deployed the most-advanced tools and state-of-the-arty technologies in order to characterize the gastrointestinal symbiotic bacteria of economically important fruit flies. Our studies were focused on the bacterial diversity, and the V3-V4 hypervariable region of the 16S *rRNA* gene was used to characterize the gut associated bacteria from laboratory, mass-reared and natural populations.

Data from sequencing barcoded PCR amplicons, coupled with classical microbiological approaches are presented and possible implications for practical applications are discussed.

Abstract ID 120

"Boosted SIT" as an Additional Tool an AW-IPM Programmes

Jérémy BOUYER

French Agricultural Research Centre for International Development (CIRAD), Montpellier, France; Insect Pest Control Unit, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria, Email: *J.Bouyer@iaea.org*

Recently, we hypothesized that shifting the vision of the sterile male from a sexual competitor only to a specific transporter of active biocides to the targeted female might boost the impact of the sterile insect technique (SIT).

In the REVOLINC (Revolutionizing Insect Control) project, we are demonstratating this concept using three biocides: Pyriproxifen, *Bacillus thuringiensis* and a Densovirus against the tiger mosquito (*Aedes albopictus*). Pyriproxifen is also tested against tsetse (*Glossina palpalis gambiensis*) and fruit flies (*Ceratitis capitata*). The technology is presently tested in the laboratory and preliminary results will be presented, as well as a model predicting the relative impacts of SIT and boosted-SIT on the dynamics of the targeted populations. The next steps will be to validate the predictions in operational field trials and compare the evolutionary response of the target populations to these different control pressures (multiple lethal mutations, multiple lethal mutations + biocides, biocides alone), for the three different biocides and three demographic strategies.

This will generate breakthrough knowledge on the transmission of biocides and pathogens in insects and the sustainability of genetic control, provide a new control technique for mosquitoes, and improve the cost-effectiveness of SIT in tsetse and fruit flies. We are also addressing technical issues associated to mass-rearing, sterilization, sex separation and aerial release of mosquitoes as well as regulatory issues required for releasing sterile males carrying various biocides.

The methodology of the project will be presented, as well as the intermediary results. The REVOLINC project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 682387).

Abstract ID 218

The Role of Drones in AW-IPM Programmes: a Drone for Sterile Tsetse Releases in Ethiopia

David BENAVENTE[1] and Rafael ARGILÉS-HERRERO[2]

[1]CEO Embention, Alicante, Spain, Email: *david.benavente@embention.com*
[2]Insect Pest Control Unit, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria

The concept of area-wide integrated pest management (AW-IPM) implies that interventions against the target pest are applied at the scale of its total population in a given area. In most cases, a pest population spreads over large surfaces, ranging from tens or hundreds of hectares in the case of some anthropophilic mosquitoes tied to human settlements to thousands of square kilometres in the case of pests with good dispersal capacity such as fruit flies or screwworms. This approach requires effective tools that can perform their assigned duties over very large areas.

Until now, light aircrafts have been extensively used in AW-IPM programmes, for the collection of relevant information (e.g. mapping of suitable habitats), aerial spraying, release of parasitoids or sterile insects. Remotely-Piloted Aircraft Systems (RPAS), commonly known as drones, are becoming very widely used not just for military but also for many civilian and commercial applications, and the technology is now mature to play a significant role in AW-IPM programmes. The state of the art of the technology, examples of current and potential uses of drones in AW-IPM programmes, as well as the restrictions imposed by the legal framework, will be discussed in this presentation.

Tsetse flies can be found in around 37 African countries. They are the carriers of parasites that cause sleeping sickness in humans and the wasting disease nagana in domestic animals. With nagana, even if animals survive, production is reduced and use of animals for ploughing or transport is not possible. Nagana costs around 4.5 billion US dollars per year in direct agricultural losses.

The Ethiopian Ministry of Livestock is currently undertaking an ambitious project to get rid of the flies from an area of 25,000 square kilometres, called “Southern Tsetse Eradication Project”. The Deme Valley, the first pilot area of the project, is now at the final elimination stage with weekly aerial releases of sterile flies from a light aircraft. An RPAS has been developed to replace the aircraft currently in use, taking advantage of the relatively low weight of insects that need to be released at low densities over large areas.

This tool consists of two main elements: a fixed-wing drone with an embedded tsetse release system and a ground control station. This 25 kg drone can fly for 3+ hours at velocities of 100 km/h. This enables the definition of long mission plans covering the affected areas. The

drone is equipped with a professional autopilot Veronte that allows not only to handle the complete mission autonomously but also to release variable rates of sterile males in precise coordinates.

As payload, the drone has 2 external pods containing a full set of small cardboxes with sterile insects inside. These pods also make room for a 7C phase-change material to guarantee optimal thermal and transport conditions for the flies.

The feasibility of the concept and the system with the actual pods and dummy cardboxes has already been proven in Spain. Currently, Aviation Authorities from all over the world are issuing regulations for drones that preserve safety for other airspace users, people and property on the ground. For this same reason, the system is awaiting a clearance from the Ethiopian Authorities to start the operation.

Session 7: Discussion Panels

Abstract ID 205

Engagement of the Public in Support of Area-wide Integrated Pest Management

Pamela PENNINGTON

Universidad del Valle de Guatemala, Guatemala City, Guatemala, Email: *pamelap@uvg.edu.gt*

The adoption of novel integrated insect pest management strategies requires proof-of-concept demonstrations. Participatory action research was successfully used to implement a pilot community-based programme for the control of insect vectors of Chagas disease in rural Guatemala.

Based on this experience, the following methodology is proposed to engage the public in support of area-wide integrated pest management (AW-IPM) programmes. To gain acceptance at the national level, the first draft of the project should be developed with input from government officials. This should include an initial field visit to gain a first-hand understanding of the problem at the local level. A situational analysis regarding current pest control policies, practices and relevant stakeholders is generated through interviews with key leaders and decision-makers, at both national and local levels (governmental and non-governmental organizations). The project´s overall aims are then presented to identified stakeholder representatives, thus gaining entry.

A generative dialogue is established with key stakeholders, jointly identifying strengths, weaknesses, opportunities and threats regarding current pest control strategies and proposing solutions through AW-IPM. Selected communities are then invited to participate in the project. Locally-relevant strategies are further adapted to implement the proof-of-concept demonstrative project. It is critical to maintain constant communication with the local and national leaders, involving them throughout the implementation and evaluation processes.

Flexibility should also be built into the project to allow for community-driven changes in the strategy, through cyclical joint process evaluation. Periodic feedback of project development should be scheduled with key stakeholders to maintain rapport. Finally, the results of the evaluation should be communicated to all stakeholders to ensure diffusion of the innovation.

Poster Presentations Session 1: Operational Area-wide Programmes

Abstract ID 2

MOSCASUL Programme in Brazil: First Steps and Current Situation

Adalecio KOVALESKI[1] and Thiago MASTRANGELO[2]

[1]EMBRAPA Uva e Vinho, Estação Experimental de Vacaria, Brazil
[2]Centro de Energia Nuclear na Agricultura da Universidade de São Paulo, Brazil, Email: *thiagomastrangelo@gmail.com*

The South region concentrates 99% of the apple growing areas of Brazil, with a harvest of more than 1.1 million tons of fruits and a supply chain moving up to USD 3 billion annually. Despite the occurrence of several species of fruit flies in the region, the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) represents 98.5% of the flies captured in commercial apple orchards. The gross value of yield losses associated with the chemical control of this pest were estimated at more than USD 25 million per year, and the number of fruits infested by *A. fraterculus* has increased during the past three years, since the most common insecticides used for its control, especially organophosphates, are being banned due to toxicological restrictions.

Seeking for environmentally-friendly alternatives to suppress the pest, such as sterile insects and parasitoids, Brazilian researchers, along with State organs and the Brazilian Association of Apple Producers (ABPM), started to plan the creation of the MOSCASUL Biological Control Center. After the support of the Ministry of Agriculture, Livestock and Food Supply (MAPA) at the end of 2014, almost USD 600,000 have been invested in building a mass-rearing facility at the Agricultural Experiment Station of EMBRAPA Grape and Wine, and the first containerized laboratory modules were installed in May 2016.

Meanwhile, a pilot trial was planned to be carried out in three large apple orchards at the municipality of Vacaria, Rio Grande do Sul State. As the Center for Nuclear Energy in Agriculture (CENA) possesses a 250 m^2 facility to mass-rear fruit flies and two gamma irradiators, the sterile flies for the pilot trial will be initially provided by CENA for the next one or two years. Both sterile flies and parasitoids will be released in buffer zones of forests located at 100-150 m of the periphery of the target orchards, since these areas serves as the source of wild flies. Based on the densities of the wild populations, about 150,000 sterile flies are required for the 1st pilot release phase. After the fine-tuning of all rearing and sterilization procedures at the beginning of 2016, CENA started shipping more than 200,000 irradiated pupae weekly by air to Vacaria for 6 months, starting on September 2016 when the level of the wild fly population is extremely low. Most of the studies on marking and shipment procedures, besides the training of the teams for surveillance, distribution of the sterile flies, and identification of the insects have been completed. The feasibility of shipping irradiated eggs for the mass-rearing of two parasitoid species at Vacaria is also being assessed. The results of this pilot trial will influence the direction of future control tests and benefit the

area-wide management of *A. fraterculus* involving almost 50 thousand apple producers and other temperate fruit growing farmers from the Southern States of Brazil.

Abstract ID 4

Detection and Monitoring of Fruit Flies (Diptera: Tephritidae) Using the Male Annihilation Technique in Central Sudan

Abdelaziz GESMALLAH

University of Gezira, Sudan, Email: *azizatniphe@gmail.com*

Fruit flies are major pests of economic importance worldwide. In Sudan, they adversely affect production and export of fruit and vegetable crops. To obtain a more comprehensive indication of fruit fly diversity, a trapping programme using McPhail traps baited with male attractants (methyl-eugenol, terpinyl-acetate, cue-lure and trimedlure) was conducted over 48 weeks from June 2009 to May 2010 at Gezira and Sennar States.

Morphological identification of the collected specimens revealed the presence of five fruit fly species, namely *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, *Ceratitis cosyra*, *C. capitata* and *C. quinaria* in the surveyed states. Two other species, *Dacus ciliatus* and *D. punctatifrons,* were detected only in Sennar State. For the species *D. punctatifrons* it is the first time to be recorded in Sudan, where 104 specimens were caught using the male attractant cue-lure.

Populations of *C. capitata* and *C. quinaria* were very low in the two states (12 and 118 specimens over the trapping period for *C. capitata,* and 146 and 34 for *C. quinaria* in Gezira and Sennar State respectively). *B. dorsalis* was present all year round in Sennar and Gezira States. Generally the population of this species was higher in Gezira (total number of 29,514/trap than in Sennar (4,778/trap).

B. dorsalis proved to be the most dominant fruit fly species in Gezira State representing 98.4% of fruit fly composition, replacing the indigenous species *C. cosyra*. Results showed that *Z. cucurbitae* was the most abundant species in Sennar State, representing 58% of fruit fly composition, followed by *C. cosyra* and *B. dorsalis*, with 20.9% and 20.5% respectively. Methyl-eugenol showed strong ability to attract males of *B. dorsalis*.

The study provides knowledge of the Tephritidae spectrum in Gezira and Sennar States, which is a prerequisite for the development of any IPM programme.

Key words: Fruit fly, attractant, biodiversity, Sudan

Abstract ID 12

Performance of Mango Fruit Fly Area-Wide Management in Central Uganda

Caroline NANKINGA, Alex MAYAMBA, Florence NAMPEERA, William WASWA, Victor LUBEGA, Andrew KIGGUNDU and Wilberforce TUSHEMEREIRWE

National Agricultural Research Laboratories, Kampala, Uganda, Email: *cmnankinga@gmail.com*

Background: In Uganda fruit and vegetable production is constrained by a multiplicity of pests and diseases, the most serious of which are fruit flies (Diptera: Tephritidae). The Oriental fruit fly, *Bactrocera dorsalis* Hendel, is the most destructive pest in mango, *Mangifera indica* L. Damage attributed due to fruit fly infestation ranges from 33 to 83% and can reach 100% if no interventions are put in place (Nankinga et al., 2015). This paper describes integrated fruit fly management options that are being scaled up using an area-wide approach to increase management effectiveness and efficiency.

Methodology: Male Annihilation Technique (MAT) in combination with mango orchard sanitation, reduced risk insecticide sprays and bagging were promoted for area-wide management of *B. dorsalis* in Nakaseke sub-county beginning March 2015. Nakaseke is a model sub-county chosen by the National Agricultural Research Laboratories to demonstrate farmer-demanded technologies for managing production constraints in fruits-vegetables, banana-coffee, maize-beans, cassava-potato farming systems.

Five hundred mango growers from Kassagga, Kyamutakasa, Kasambya and Kigeege parishes were trained on the advantages of deploying area-wide management in suppressing fruit fly populations as opposed to single option orchard management. The trained farmers were supplied with 2000 MAT blocks containing methyl-eugenol (4-allyl-1,2-dimethoxybenzene-carboxylate) plus malathion, dispensed in locally fabricated traps made from 3 litre yellow plastic jericans randomly distributed on approximately 500 acres of mangoes intercropped with other crops.

Results: Farmers who installed traps at the beginning of the season and practiced orchard sanitation reported reduced mango fruit fly infestation. Farmers who had abandoned exotic mango orchards registered their first sells in the March-June 2015 fruiting season and were excited with enormous *B. dorsalis* male fly trap catches (>10,000 male flies/trap in 3 weeks). Farmers in Kyamutakasa and Kasambya parishes reported more than 50% increase in volume of mangoes sold as a result of reduced mango fruit fly infestation. Three farmers who practiced bagging of the exotic Kent, Tommy Atkins mango varieties harvested 100% fruit fly free mangoes by the end of the season.

Mangoes picked from 50 randomly selected mango orchards showed reduced fruit fly infestation (from 61 pupae/kg to 7 pupae / kg of fruit) after deployment of the fruit fly control interventions. Success stories of using of MAT blocks in fruit fly suppression were exhibited

in two agricultural community shows and the technology is being scaled out through farmer to farmer communication channels and community radios.

Conclusion: A combination of Male Annihilation Technique, mango orchard sanitation, bagging and reduced risk insecticide sprays, under area-wide management, reduced fruit fly infestation in mangoes in central Uganda. Efforts to raise awareness concerning the benefits and impact of fruit fly area-wide management should be supported to increase the adoption of area-wide management in other regions in the country.

Key words: Bactrocera dorsalis, area-wide, methyl eugenol, sanitation

Abstract ID 24

SIT Application in Spain: AW-IPM Programme against Mediterranean Fruit Fly and Ongoing Activities for Future Implementation of SIT over Mosquitoes

Ignacio PLÁ-MORA and Carlos TUR-LAHIGUERA

Empresa de Transformación Agraria S.A. (TRAGSA), Ministry of Agriculture, Spain, Email: *ipla@tragsa.es*

The Sterile Insect Technique (SIT) is an increasingly important component of area-wide integrated pest management programmes (AW-IPM) for key insect pests. In Valencia Spain, an area-wide programme with an SIT component, managed by TRAGSA, is implemented since 2007 to suppress the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). Currently this environmentally-friendly technique is being considered as a potential tool for application against mosquitoes.

The Mediterranean fruit fly is an endemic pest in the fruit growing areas along the Mediterranean coast of Spain affecting many fruits like citrus, kaki, peach, apple, etc. In Spain the main damaged crop is citrus, since our country is the main producer of citrus in the EU and the fifth in the world. The citrus is mainly grown along the Mediterranean coast, being the Valencian Region the main production area from where more than a half of the national production is obtained. This region is the main exporter in the world of citrus for fresh consumption, being the Mediterranean fruit fly a quarantine pest in several destination countries. For more than fifty years an AW-IPM programme against the Mediterranean fruit fly has been implemented in the Valencian Region, being the SIT the main component during the last decade.

A mass-rearing facility was built in 2006 with a maximum production level of 500 million sterile pupae per week. The sterile males are released over more than 150 000 hectares of citrus crop. Through a monitoring network installed throughout the working area, the population level is known and the most adequate suppression tools are applied in the different areas: mass-trapping, ground and aerial chemical treatments (special authorization for specific season and areas), sanitation, distribution of traps to the farmers, and installation of traps on isolated hosts.

In terms of mosquitoes, a considerable increase in the spread of invasive species has been observed within Europe since the late 1990s, with the Asian tiger mosquito *Aedes albopictus* continuously expanding its distribution. It is now present along the whole Mediterranean coast of Spain and some regions in the north of the country. Requests for exploration of the potential for applying the SIT against mosquitoes as part of area-wide integrated vector management programmes (AW-IVM) continue increasing.

There are many aspects of the mosquito SIT package that require research for increased efficiency at the operational level. Since 2013, TRAGSA is working on several of them collaborating in Coordinated Research Projects coordinated by FAO/IAEA to develop innovative equipment to improve mass-rearing of disease-transmitting mosquitoes. It includes adult mass-rearing and only (sterile) male cages, pupae sex sorter (including female removal) and male transportation and release systems.

During the next years several activities and a SIT pilot project are planned in the Valencia Region. During the first year, surveillance, dispersal trials, competitiveness tests in laboratory and semi-field cages, amplification of *Ae. albopictus* colony and public awareness activities are planned. For the second year the SIT pilot project against *Ae. albopictus* will be implemented. TRAGSA counts on FAO/IAEA technical support in all the previously detailed activities through the renewed Practical Arrangement signed by two parties in April 2015.

Abstract ID 29

Population Density and Distribution of Mediterranean fruit fly, *Ceratitis capitata* in Orchards of the Middle Region of Iraq

Mohammed KHALAF and Hussain ALRUBEAI

Integrated Pest Control Research Center, Directorate of Agricultural Research, Baghdad, Iraq, Email: *mkhalaf34@yahoo.co.uk*

Field experiments were conducted during 2013 to measure the population density and distribution of Mediterranean fruit fly, *Ceratitis capitata*, in apricot and citrus orchards of Baghdad and Wasit governorates. The field surveys were done in four orchards (1 ha. each), two of them in each governorate, one for apricot and other for citrus. To monitor the density, Delta and McPhail traps supplied with the male and female attractant pheromones and insecticide strip, were distributed in each orchard.

The results indicated that population density of the Mediterranean fruit fly was higher in Wasit orchards than in Baghdad orchards. The peak density in apricot orchards occurred during the last week of May and first week of June, coinciding with fruit ripening. In citrus orchards the peak density was in November in both regions. Fruits infestation percentage indicated that early ripening fruit varieties of apricot were less infested than late varieties. In citrus, mandarin was found the most favourable to infestation by Mediterranean fruit fly, followed by orange, then sour orange and lemon fruits.

These results are important for the integrated control programmes of Mediterranean fruit fly, eventually including the possible application of Sterile Insect Technique (SIT).

Abstract ID 32

Towards the Control of *Bactrocera oleae* Gmelin (Diptera: Tephritidae) Using Sterile Insect Technique in Iran

Mehrdad AHMADI, Bahareh SALEHI and Mohammad BABAIE

Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Teheran, Iran, Email: *mehrdada111@gmail.com*

Background: The olive fruit fly is one of the most harmful pests in olive plantations in a number of provinces in Iran. Heavy olive fly infestation can decrease the quality and therefore the value of the olive oil by up to 80%, and cause the refusal of total products of table olives.

The Sterile insect technique (SIT) is an ecologically-friendly and species-specific system of pest control, which is based on the release of large numbers of sterilized insects. In this technique, the number of fertile matings is reduced by a competition for mating between the wild and sterile released males, which finally induces a decline in the whole population size. SIT can be effective for pest management; unlike chemical control it has no off-target effects on the environment or on human health.

Methodology: The laboratory culture of olive fruit flies originated and was established from infested olive fruit in Roodbar, Manjil, Rostam Abad, Tarom and Qazvin in Iran. To determine the sublethal doses of gamma radiation, 3-5 days old pupae were irradiated by 0, 50, 70, 90, 100, 120, 140 and 160 Gy doses.

Results: The statistical analysis shows a significant decrease in the mean number of eggs laid by non-irradiated females crossed with irradiated males. The egg hatching percentage declined markedly with the increase of radiation dose and no eggs hatched in the 100-160 Gy dose range. The sterility was found to be completely correlated with the dose, being 64.19, 74.78, 94.56, 100, 100, 100 and 100 % at the doses of 50, 70, 90, 100, 120, 140 and 160 Gy, respectively. The results indicated that the gamma radiation caused significant reduction in adults mating rate, female fecundity and egg hatching of *B. oleae*.

Conclusion: According to these results and the data from laboratory bioassays, the correlation between sterility and insect competitiveness is provided in the range of 90 and 100 Gy, indicating the best irradiation dose of *B. oleae* pupae.

Abstract ID 41

Backyard Mediterranean Fruit Fly is a Key Factor in Area-Wide Management in Southern Europe. Data from Attiki Greece, 38 North Latitude

Aristidis ECONOMOPOULOS[1] and Polychronis REMPOULAKIS

[1]Professor emeritus, University of Crete, Greece, Email: *economop@biology.uoc.gr*

Background: For the cosmopolitan multi-host Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera; Tephritidae), the development of an effective area-wide control system is an essential prerequisite for the successful handling of populations from numerous city and suburban small orchard and back-yard fruit trees. These numerous breeding places, difficult to handle for many reasons, can produce massive population outbreaks, which will not allow effective control in the continuum of fruit tree plantations in the vicinity of small or large cities and suburbs.

Methodology: A twelve-month back-yard fruit tree Mediterranean fruit fly record was kept from November 2015 till November 2016, in a small back-yard fruit-tree garden about 7 km from the center of Athens. McPhail yellow bottom plastic traps (one per tree) with BioLure (3 separate dispensers of ammonium acetate, trimethylamine, and putrescine) and a water solution of 1.5% sodium tetraborate and few drops of kitchen detergent were used. Borax was not added during the winter months. Traps were examined approximately every week, except winter when examination periods were longer, and water solution renewed. The BioLure dispensers were renewed every 3-4 months.

Results: In a navel orange tree, a few medflies (3.6-5.3/day) were trapped in November, less than 1 Mediterranean fruit fly/day was trapped between December and May, except mid-April when 2.1 flies/day were trapped. From June till October high to very high numbers were recorded. In June-July records up to more than 90 flies per day were recovered from the trap. In August-October records up to 66 flies per day were captured. The sex ratio of trapped flies was almost always in favour of females, usually between 60-100%. An increase of Mediterranean fruit fly catches was always observed following BioLure dispensers renewal.

Conclusion: Given the huge number of fruit-tree small gardens in and around small or big cities in the Mediterranean, the common lack of insect control practices in those areas, and the frequent continuum of extensive commercial fruit tree plantations beyond the suburban areas, we suggest that no area-wide *C. capitata* management should be undertaken unless all these breeding areas are addressed. In case SIT integration is considered, the reproductive behaviour of released flies in this special environment must be thoroughly investigated, i.e. very small and fragmented host tree plantations with many buildings and ornamental plants in-between, continuous illumination from street, security, and house lights, etc.

Abstract ID 44

Eradicating Mediterranean Fruit Fly from Carnarvon, Western Australia Using Area-wide Management, Community Engagement and Sterile Male Release

Bill WOODS and Roselia FOGLIANI

Department of Agriculture and Food Western Australia, Perth, Australia, Email: *bill.woods@agric.wa.gov.au*

The Mediterranean fruit fly is established in orchards, vineyards and in urban and peri-urban areas in Western Australia. It is a major pest in the isolated Carnarvon horticultural area 900 km north of Perth, which produces fruits, table grapes and vegetables, including capsicums and chillies. This coastal town (population 6,000) has a warm semi-arid climate (average minimum 11-23^{o}C, average maximum 22-33^{o}C, and average yearly rainfall 230 mm) and is surrounded by rangeland without fruit fly hosts. The Carnarvon area has had a fruit fly baiting scheme running for over 50 years, which has kept fruit fly numbers to a manageable level, where effective control could be obtained using cover sprays of either fenthion or dimethoate. The recent restriction of use of these products has placed an increased emphasis on the need for new area-wide approaches to maintain effective fruit fly control.

In 2015, a three year campaign to eradicate Mediterranean fruit fly from Carnarvon commenced, funded by the state government of Western Australia through its Royalties for Regions Programme, Horticulture Industries Australia, the Carnarvon Growers Association (CGA) and the Shire of Carnarvon. From the beginning of this campaign it was realised that strong community engagement would be required for the programme to be successful. A Community Engagement Biosecurity Officer was employed to liaise closely with the local industry and individual growers, many of which are Vietnamese. The use of fruit fly traps on all properties has been an effective engagement tool and a plantation hygiene compliance plan has been completed and implemented. This is particularly important in crops like chillies and capsicums, which if left unmanaged, can breed large numbers of fruit flies, which then move to adjacent fruit fly host crops. The baiting scheme managed by the CGA is a critical component of the eradication campaign if fly numbers are to be reduced to a level where SIT can be effective. Foliage baiting is augmented with mass-trapping and attract-and-kill in urban and peri-urban areas.

Two to five million pupae (VIENNA 7 genetic sexing strain) are produced in Perth and irradiated at 100-120 Gy in a RadSource x-ray irradiator under hypoxia and sent by road overnight to Carnarvon where they are emerged in Worley towers in a purpose built release facility. Flies are treated with ginger root oil aromatherapy before release by a chilled fly release machine, which releases flies at a target rate in an area defined by GIS coordinates. GIS systems and ground-truthing have been used to record and map fruit fly hosts within the

Carnarvon growing area. An extensive trapping grid of over 200 Jackson traps has been established in the town and plantation area. Trap bases are checked fortnightly under epi-fluorescent microscopy to record progress of the eradication. A targeted awareness campaign for travellers, road signage, gazetted fruit movement restrictions and mobile roadblocks will be used to maintain area freedom.

This paper reports on progress to date of the eradication, problems encountered and likelihood of success.

Abstract ID 45

Assessing the Efficacy of Integrated Pest Management Strategies for Suppressing *Bactrocera dorsalis* in Selected Orchards in Manica Province, Mozambique

Domingos CUGALA and Albertina MAJACUNENE

Eduardo Mondlane University, Maputo, Mozambique, Email: *dcugala@gmail.com*

Background: The invasive fruit fly, *Bactrocera dorsalis*, was first detected in Mozambique in 2007 (Cuamba District, Niassa Province) and is now well established and widespread in the northern and central regions of the country. Direct damage to mango and guava due to *B. dorsalis* has been reported to range from 56.5% to 92.5% in the northern province of Cabo Delgado. In addition to the direct losses, indirect losses due to quarantine restrictions have been enormous since the occurrence of *B. dorsalis* has led to the suspension of fruit and vegetable exports to the country's major trading partners.

To overcome the impact of *B. dorsalis*, in Mozambique, the use of integrated fruit fly management strategies for population suppression has been applied by farmers, combining GF-120 bait spray, HymLure (food attractants combined with Malathion insecticide), male annihilation and orchard sanitation.

Methods: A total of 8 commercial farms were included, of which 4 apply IPM strategies, while in the other 4 no control measures were applied. The efficacy of IPM measures was assessed by comparing *B. dorsalis* population between the IPM-treated farms and the non-IPM treated ones (control plot). Adult *B. dorsalis* population size was monitored on a monthly basis using methyl-eugenol-baited traps.

Results: During the 2012-2013 season, the mean population density in the IPM-treated farms was 5.7 flies / trap / day (FTD), much lower than the 149.3 FTD in the control farms, corresponding to a 96.2% population reduction on the IPM-treated farms.

Conclusion: These results suggest that *B. dorsalis* populations can be significantly suppressed through a combination of management measures in an orchard, and this combination of IPM strategies could be exploited for a large-scale application in the country.

Key words: Fruit flies, *Bactrocera dorsalis*, IPM, area-wide control

Abstract ID 46

Evaluation of the Efficacy of a Commercial Formulation of *Beauveria bassiana* for the Control of the Invasive Fruit Fly *Bactrocera dorsalis* (Diptera: Tephritidae)

Dinah MARRI and Michael OSAE

Ghana Atomic Energy Commission, Accra, Ghana, Email: *dinah.marri@yahoo.com*

Background: Bactrocera dorsalis (Diptera: Tephritidae) is a major quarantine pest devastating the fruit and vegetables sector in Ghana and most African countries. Current control strategies have not been able to reduce their impact significantly. In the quest for novel control tools, a commercial formulation of *Beauveria bassiana* (Botanigard® ES) was evaluated against the pest.

Methodology: Evaluation of the efficacy of Botanigard® ES against *B. dorsalis* was carried out on three developmental stages. Botanigard® ES containing 11.3% *B. bassiana* GHA strain was applied to larvae, pupae and adults at concentrations of 106, 53.0, 26.5, 13.3 and 6.65 ($\times 10^6$ spores/mL).

Results: The effect of Botanigard® ES on *B. dorsalis* was dose dependent, increasing with fungal spore concentration. The results showed that an optimum dose of 26.5×10^6 spores/mL killed 50% of adult flies within 4–5 days and 99% within 8–9 days. The same dose reduced adult emergence from infected pupae from 97% in the control to 46% in the treated groups. Fungal treatment at 26.5×10^6 spores/mL also killed almost all adults emerging from pupae and adults treated directly.

Conclusion: The result indicated that applying Botanigard® ES at a dose of 26.5×10^6 spores/mL in fruit fly traps in mango canopies is a better method for fruit fly control in the field as compared to soil surface spray method. However both methods could be used simultaneously for better results.

Abstract ID 49

Fruit Fly Research and Development in Africa - Towards a Sustainable Management Strategy to Improve Horticulture

Sunday EKESI[1], Samira MOHAMED[1] and Marc DE MEYER[2]

[1]International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya
[2]Royal Museum for Central Africa, Tervuren, Belgium, Email: *demeyer@africamuseum.be*

Background: The horticultural sub-sector presents many opportunities for economic development and improving livelihood of millions of growers of fruits and vegetables and other stakeholders. However, several factors constrain production and limit the potential for trade of these commodities. Tephritid fruit flies (Diptera: Tephritidae) constitute a major constraint. They cause enormous losses through direct feeding damage and loss of market opportunities through imposition of quarantine restrictions by importing countries to prevent entry and establishment of invasive species. In Africa, several native (*Ceratitis* and *Dacus*) and exotic (*Bactrocera* and *Zeugodacus*) species inflict considerable losses to horticulture ranging from 30 to 90%, thereby threatening the livelihood of vulnerable rural communities who rely exclusively on agriculture for their income generation, as well as jeopardizing the African economy at large.

Methodology: Over the past 20 years of R&D on the African mainland and adjacent islands, extensive information has been generated on several native and exotic fruit flies. Numerous studies have been conducted and papers published in several fields of taxonomy, biology, ecology and management. This output coincides with two elements: the changing fruit fly landscape in Africa – caused by arrivals of the highly destructive alien invasives (*Bactrocera dorsalis, B. zonata,* and *B. latifrons*) – and the priorities that African countries have placed recently on export of fruits and vegetables to international markets. As such it was deemed necessary that all this scattered information should be brought together in a reference work that collates the series of important and diverse achievements made over the last two decades.

Results: Sixty-five scientists from Africa and abroad present in 34 chapters the current state of the art in these different fields. They analyse the successes achieved in the identification of different species using both morphological and molecular tools. Also, the invasion histories of exotic species are documented, and information on behaviour, abundance, dynamics, host plants and damage levels of different species are presented. Management methods based on the use of baiting and male annihilation techniques, biopesticides, parasitoids, ant technology and field sanitation are discussed in line with the demand for socio-economic impact and ecosystem sustainability. The technical knowledge presented in this book is not unique to Africa, and lessons learnt from other successful fruit fly eradication/management programmes in the USA and Latin America are also captured.

Conclusion: As such it is hoped and expected that this work will serve as a reference tool for the current and future generation of fruit fly researchers in Africa.

Abstract ID 50

Strengthening Fruit Fly Research between Europe and Africa through Networking

Pia ADDISON[1], Domingos CUGALA[2], John-Henry DANEEL[3], Hélène DELATTE[4], Marc DE MEYER[5], Pierre-François DUYCK[4], N'klo HALA[6], Minette KARSTEN[7], Caroline KNOX[8], Aruna MANRAKHAN[3], Maulid MWATAWALA[9], Massimiliano VIRGILIO[5] and Christopher WELDON[10]

[1]Stellenbosch University, Stellenbosch, South Africa
[2]Eduardo Mondlane University, Maputo, Mozambique
[3]Citrus Research International, Nelspruit, South Africa
[4]CIRAD, St Pierre, La Réunion, France
[5]Royal Museum for Central Africa, Tervuren, Belgium, Email: *demeyer@africamuseum.be*
[6]Centre National de Recherche Agronomique, Abidjan, Côte d'Ivoire
[7]Stellenbosch University, Stellenbosch, South Africa
[8]Rhodes University, Grahamstown, South Africa
[9]Sokoine University of Agriculture, Morogoro, Tanzania
[10]University of Pretoria, Pretoria, South Africa

Background: Europe and Africa have a common interest in fruit fly research. This is because of invasive species risks, agricultural trade and overlap in cultivated crops, but also due to historic reasons and geographic proximity. In order to facilitate collaboration and exchange of information, networking projects with partners in Europe and Africa were initiated since 2014.

Methods: ERAfrica FRUIT FLY is co-ordinated by Citrus Research International, South Africa. Its main objectives are improving detection and monitoring methods for fruit flies, and identification of African fruit fly pests. Partnering institutions in the project are CIRAD in Reunion, France, Centre National de Recherche Agronomique in Ivory Coast and the Royal Museum for Central Africa (RMCA), Belgium. Trapping surveys with new and standard attractants are being conducted in South Africa and the Ivory Coast. The CIRAD team focuses on screening fruit volatiles for monitoring of female flies with poor response towards currently available attractants. The RMCA team makes use of data obtained to test, modify and improve existing identification tools.

FRUITFLYNET: Monitoring network for fruit flies in south-eastern Africa is funded by the Belgian Science Policy and co-ordinated by the RMCA. Partnering institutions are Stellenbosch University in South Africa, Sokoine University of Agriculture in Tanzania, and E. Mondlane University in Mozambique. All have ongoing fruit fly research activities in their own countries which are diverse in nature. The main objective is to harmonize the different activities, and facilitate further interactions among them. As there are overlapping interests in both networks, there is a regular exchange of information with the aim of developing

overarching initiatives. In addition, there is also exchange of information with other international networking activities in Africa.

Results: Over the last two years, the different partners have met in separate or joint meetings. Partners of both networks organized the third international symposium of fruit fly researchers of Europe, African and the Middle East (Stellenbosch 2016). Numerous significant results that were reached over this period, were presented at this and other international gatherings and have been published.

Conclusion: Further initiated joint activities are the development, with other stakeholders, of a project proposal on establishment and maintenance of fruit production areas free and under low prevalence of fruit fly pests in southern Africa, and of a mobile app allowing identification of fruit flies of economic significance and access to all relevant information of occurrence, hosts and management.

Abstract ID 51

Development and Implementation of Area-wide Fruit Fly Control in Chili Crops in West Java, Indonesia

Tati Suryati SYAMSUDIN[1], Rinda KIRANA[2], Aos AOS[1] and Rika ALFIANNY[1]

[1]School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia, Email: *tati@sith.itb.ac.id*

[2]Indonesian Vegetable Research Institute, Department of Agriculture, Indonesia

Background: Indonesia is the country with the second largest area of chili pepper production covering 232,807 ha; however, chili pepper productivity is the lowest (about 7.4 ton/ha). As a multifunction commodity, chili has an important role in daily lives of the Indonesian community. It consumes them fresh as spices, and as raw materials for industry (medicals, cosmetics, etc.).

One of the problems in chili production is cultivation in scale areas, with small capital and land use competition with the main crops. In Indonesia Integrated Pest Management (IPM) has been introduced three decades ago and it is focused on main crops (such as rice), however little attention has been paid to horticultural commodities. The objective here was to develop and implement area-wide fruit fly control in chili crops.

Methods: Development of fruit fly control on chili was conducted by Male Annihilation Technique (MAT) using methyl-eugenol traps at an agriculture experimental station (40 ha) at the Vegetable Research Institute in Lembang, West Java. A total of 30 baited traps were set in chili crops and fruit flies were caught during 7 weeks. Implementation of fruit fly control programme on chili was conducted at two locations: at Bandung regency (50 ha) and Sumedang regency (40 ha). The programme involved 60 local farmers, which met and discussed in the form of two Forum Group Discussions. Dissemination on the knowledge of chili damages and its pest was introduced in these Forum Group Discussions. The field works was conducted by setting the traps in chili crops. Forty five baited traps were distributed and set at each location. Programme evaluation was done by verification of fruit flies caught and discussion between local farmer groups.

Results: The experiment at the Vegetable Research Institute on chili crops showed that MAT was very effective using 1 ml Petrogenol per trap. Lack of information on fruit fly attack on chili fruits caused unfriendly pest control on chili fruits by the farmer. Previously, the local farmers controlled chili pests using insecticides, fungicides (non-selective control with uncertain dose) and there were no specific actions for chili fruit fly control. The farmers didn't know that the cause of chili damage was fruit flies.

Implementation of fruit fly control programme on chili was started by introducing the knowledge of chili pest and new control method using attractant. The results of trapping

methods in chili crops showed that the number of fruit fly (*Bactrocera dorsalis* complex) caught decreased from 8 to 2 flies/trap/day.

Conclusion: Fruit flies caught in each trap convinced the local farmers to control chili fruit fly. These results also showed that the sustainability of the chili fruit fly control programme depends on the informal leader in Forum Group Discussions.

Abstract ID 56

Establishing an Area of Low *Bactrocera* (*invadens*) *dorsalis* Prevalence in Kenya

Muo KASINA[1], Joseph MULWA[1], Peter MAINGI[2], Teresia KARANJA[3], Grace KYALLO[4], George MOMANYI[5] and Matolo NYAMAI[2]

[1]National Sericulture Research Centre, Kenya Agricultural & Livestock Research Organization (KALRO), Thika Kenya, Email: *muo.kasina@kalro.org*
[2]Kenya Agricultural and Livestock Research Organization, Kabete, Kenya
[3]Plant Protection Services Division (MOALF)
[4]Horticultural Crops Development (MOALF)
[5]Kenya Plant Health Inspectorate Service

Background: Fruit flies (family Tephritidae) are some of the most important pests of fruit and vegetable crops. In Kenya, they are known to cause huge farm losses, post-harvest market losses and have contributed to reduced access to external fruit and vegetable markets. The advent of *Bactrocera dorsalis* (previously *B. invadens*) in the early 2000s changed the status of fruit fly pests in Kenya. By 2008, the country had lost major fruit markets as a result of quarantine measures taken by importing countries. Kenya was required to create pest free areas (PFA) or areas of low pest prevalence (ALPP) to continue accessing these markets.

A national taskforce on fruit fly control was established to seek ways of opening and sustaining such markets through properly instituted ALPP. The team identified Kandara, which has the highest concentration of small-scale farmers growing exported avocado in the country. As any other part of the country, a mixed cropping system is common and patches of uncultivated areas exist, with varied wild hosts. Thus it was essential to adopt an area-wide integrated pest management approach.

Methodology: A delimitation study was carried out in July 2013 to identify and create boundaries of the target area. This was done using para-pheromone (methyl-eugenol) trap laced with killing agent (Malathion). Traps were placed along transect lines to cover the whole sub county. After the determination, mapping with GPS followed, covering the whole identified area, from September 2013.

Mass-trapping and use of male annihilation technique (MAT) blocks was initiated to cover the area. In addition, extensive training of farmers and community leaders was initiated to get everyone to participate and employ other practices, particularly the cultural practices in managing the pest. Further, the policy informers were kept in full knowledge about the activity.

Results: By February 2014, the activity had resulted in less than 0.1 fruit flies/trap/day (FTD) implying that it was possible for the country to achieve the desired status to export fruits. In addition, the area-wide approach to the fruit fly control provided the community with positive

externalities, with farmers reporting benefits to other non-target crops such as mango. There was no emergence of *B. dorsalis* from avocado fruits collected for larval monitoring.

Conclusion: This study was the first attempt in Kenya to create an ALPP and it was successful. We show the importance of area-wide integrated management of fruit flies in Kenya and provide other progressive fruit fly activities that have been initiated as a result of the effectiveness of this activity. We discuss key challenges that we experienced and that may prevent the country from achieving desired results particularly on aspects of sustainability. In particular, we discuss the role of the farming systems and farmer profiles in the implementation of an effective fruit fly management plan for the country.

Abstract ID 77

Suppression of Invasive *Bactrocera dorsalis* (Diptera: Tephritidae) by Using an Integrated Pest Management Approach in Zanzibar

Zainab ABDULLAH and Tatu ALI

Plant Protection Division, Zanzibar, Tanzania, Email: *zgurnah@hotmail.com*

Background: Tephritid fruit flies are a major threat to horticultural production in Zanzibar. The invasive *Bactrocera dorsalis* (Diptera: Tephritidae) is highly destructive. It poses a threat in mango production and causes devastating losses to small-holder farmers. Gulf and European countries have imposed strict trade restrictions on mango consignment, which represents a threat to export traders, mango stakeholders, and the income and food security of many rural households. The Ministry of Agriculture empowered the community of farmers to conduct a suppression programme in mango orchards by adopting an Integrated Pest Management (IPM) approach to control the fruit fly.

Methodology: The IPM practices employed were orchard sanitation, Male Annihilation Technique (MAT), food baits, early harvesting of fruits, cover sprays, and fruit sampling, in which infested fruits were collected and incubated in the laboratory for monitoring the infestation level. The programme was tested in 10 villages in the south region from 2012 - 2014.

Results: Promising results were observed. The level of infestation measured by fruit sampling was reduced from 85 to 25%. The *B. dorsalis* population was decreased by using baits, from 7.8 fruit flies/trap/day (FTD) to 1.9 (FTD) during the major mango season. During the lower mango season it declined from 1.8 (FTD) to 0.5 (FTD).

Conclusion: This is a pilot suppression programme that was proven to be effective. For this programme to be sustainable, there is a need to expand these practices to an area-wide IPM programme involving farming community groups in other regions of Zanzibar.

Key words: Sustainable, area-wide, IPM, invasive, pilot suppression, MAT

Abstract ID 83

Cold Storage of Queensland Fruit Fly Pupae for Mass-rearing Programmes

Maurizio BENELLI, Urvashi LALLU, Kate MITCHELL, Fleur PONTON and Phillip W. TAYLOR

Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia, Email: *maurizio.benelli@students.mq.edu.au*

Fruit flies (Diptera: Tephritidae) have a destructive impact on agriculture in many countries, including Australia. Native to eastern Queensland and north-eastern New South Wales, *Bactrocera tryoni* (Queensland fruit fly) is the most damaging insect pest of fruit crops in Australia. The Sterile Insect Technique (SIT), which aims to suppress wild populations by release of mass-reared sterile males, is being developed as a principal control strategy for integration in *B. tryoni* management. SIT relies on mass-production facilities to supply massive numbers of high-quality sterile male flies.

However, mass-reared insects usually have a short shelf life, needing to be used almost immediately after production. Considering the complexity of synchronizing field releases during large pest outbreaks in SIT programmes, an increased shelf life with no (or minimal) loss of post-release performance is desirable. Cold storage is a valuable tool for prolonging the developmental time of insects by exposing them to sub-optimal temperatures. Protocols have, however, not yet been established for *B. tryoni*.

The aim of the study was to evaluate cold storage of *B. tryoni* pupae under different sub-optimal temperatures and measure the effects on the emergence rate and the quality of the resulting adults. In a first test, *B. tryoni* pupae were kept at 13, 15, 17, 19 and 25°C (control) with 65% RH and complete darkness until adult emergence was completed. The emergence rate (94% for control flies) was reduced to 52% when flies were stored at 17°C and dropped to zero at 13°C. The highest percentage of partially emerged adults was found at 17°C.

In a second test, a new range of storing temperatures was used, from 17°C (the lower bound limit found with the previous test) to 19, 21, 23 and 25°C (control). One day before emergence, pupae were transferred to the standard rearing temperature of 25°C. Quality of the adults was assessed using the standard FAO/IAEA/USDA flight ability and chill coma recovery tests.

The results of this study form a basis for the use of cold storage in *B. tryoni* mass-rearing facilities, and open new avenues for further investigation on the physiological mechanisms taking place during cold storage.

Abstract ID 86

Field Evaluation of Novel Male Lure "Attract and Kill" Stations for Area-Wide Suppression of Fruit Flies (Diptera: Tephritidae) in Hawaii

Roger VARGAS[1], Steven SOUDER[1] and Ronald MAU[2]

[1]USDA-ARS, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, Hilo, Hawaii, USA, Email: *roger.vargas@ars.usda.gov*
[2]Department of Plant and Environmental Protection Sciences, University of Hawaii, Hawaii, USA

Background: In area-wide IPM control systems where fruit fly populations are large, Male Annihilation Technique (MAT) has been used as an effective suppression technology. Before the Hawaii Fruit Fly Area-Wide Pest Management [AWPM] programme, no fruit fly lures were registered for general control of fruit flies in the United States. This community-based programme registered new technologies for farmers and homeowners and promoted the use of safer or reduced risk fruit fly protein baits and MAT traps or novel dispensers in what became popularly referred to as the 1 (sanitation), 2 (protein bait), 3 (male lure trapping) programme for fruit fly control. MAT involves mass-trapping using the male lures methyl-eugenol [ME], cue-lure [C-L]/raspberry ketone [RK], and trimedlure [TML] with an approved killing agent and dispenser (e.g., SPLAT), or with certain traps with no insecticide at all. Although formal funding of the Hawaii fruit fly AWPM programme ended in 2008, research, development and transfer of AWPM components and technology have continued locally, nationally, and internationally through 2017.

Methodology: Three separate "attract and kill" male lure field trials were evaluated for oriental fruit fly (*Bactrocera dorsalis*), melon fly (*Zeugodacus cucurbitae*) and Mediterranean fruit fly (*Ceratitis capitata*), respectively. STATIC-ME-Spinosad was tested in papaya orchards to suppress oriental fruit fly on Hawaii Island. Amulet-C-L was tested against melon fly in melon fields on Oahu Island. Finally, solid TML wafers without insecticides inside traps were tested alone and in combination with BioLure mass-trapping to evaluate suppression in a coffee plantation on Kauai Island.

Results: Some of the results were surprising: 1) Very high densities (50 stations/ha) of STATIC-ME-Spinosad had to be applied for suppression of large oriental fruit fly populations, 2) Amulet C-L not only suppressed male melon flies but also female melon flies, and 3) Captures of Mediterranean fruit fly were not higher in plots treated with combinations of BioLure + TML, compared to individual components (BioLure (standard) or TML. All three male lure "attract and kill" dispensers were effective in suppressing fruit flies and there were unexpected results on females with Amulet C-L and TML dispensers.

Conclusion: These strategies and formulations deserve consideration as viable components of Area-Wide IPM systems for oriental fruit fly, melon fly and Mediterranean fruit fly,

respectively, in addition to 1) Sanitation, and 2) GF-120 Naturalyte fruit fly bait, which are currently recommended for control of fruit flies in Hawaii.

Abstract ID 89

The Area-wide Spatio-temporal Distribution of *Ceratitis capitata* (Wiedemann) Populations in the Western Cape, South Africa

Francois BEKKER[1], Matthew ADDISON[2], Adriaan VAN NIEKERK[1] and Pia ADDISON[1]

[1]Stellenbosch University, Stellenbosch, South Africa, Email: *gulu@sun.ac.za*
[2]HortGro Sciences, Stellenbosch, South Africa

Background: Area-Wide pest control is expensive to implement and therefore it is important to investigate ways in which to optimize and improve the application and impact of these programmes. Effective planning and implementation of management actions is of vital importance, be it SIT or aerial application of bait sprays.

Methodology: The area-wide spatio-temporal dynamics of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), were investigated to evaluate the effect of landscape elements and a variety of environmental factors, on the pest distribution. We utilized fruit fly monitoring data obtained from a local area-wide fruit fly control programme's trapping network, which covers two heterogeneous fruit production areas. We used geographic information systems (GIS) together with a variety of spatial analysis techniques and geostatistical procedures to describe the spatio-temporal distribution of the pest population and to investigate possible relationships between the fruit fly distribution patterns, landscape elements and environmental factors.

Some studies have looked at spatial patterns of *C. capitata* within the landscape. However, very few studies have explored the spatio-temporal patterns of *C. capitata* on such a large-scale, as was done in this study.

Results: Results show that during periods of high population numbers, fruit fly "hotspots" are observed in areas of close proximity to urban areas. However, the underlying mechanisms that might explain these "hotspots" are still to be investigated.

Conclusion: The outcomes of this research, aim to assist managers of area-wide fruit fly programmes in their planning and application of their management actions, in order to improve the effectiveness of the control programme.

Abstract ID 97

Developing Effective Detection Tools for Afrotropical Fruit Fly Pests - The ERAfrica 'FRUIT FLY' Project

Aruna MANRAKHAN[1], John-Henry DANEEL[1], Marc DE MEYER[2], Massimiliano VIRGILIO[2], DUYCK, Pierre-Francois[3], Helene DELATTE[3] and Francois Nklo HALA[4]

[1]Citrus Research International, Nelspruit, South Africa, Email: *aruna@cri.co.za*
[2]Royal Museum for Central Africa, Tervuren, Belgium
[3]CIRAD, French Agricultural Research Centre for International Development, Montpellier, France
[4]Centre National de Recherche Agronomique, France

Fruit fly pests heavily impact production and trade of fruit and vegetables in the African region. If not properly managed, fruit fly pests not only result in significant yield losses in commercial fruit and vegetable production, but they also can lead to restrictions in export trade of fruit and vegetable commodities. Many fruit fly pests in the African region are listed as quarantine pests in Europe, which is one of Africa's biggest trading partners with regards to fruit and vegetable commodities. Fruit and vegetables exported to Europe would therefore need to be free of quarantine fruit fly pests. The use of insecticides for fruit fly control is limited due to low tolerance of residues in fruit and vegetables. As such, an integrated management approach combining effective detection and control should be followed.

The ERAfrica 'FRUIT FLY' project, a joint Africa and Europe partnership project, initiated in 2014 aims to develop effective and accurate detection methods for fruit fly pests in the Afrotropical region. The project assembles a team of fruit fly experts from South Africa (*Citrus Research International*), Côte D'Ivoire (*Centre National de Recherche Agronomique*), Belgium (*Royal Museum for Central Africa*) and France (*CIRAD-Reunion*) to address different aspects of fruit fly detection systems. Fruit fly detection in the field involves the use of traps and attractants which target the adult stages of these insect pests. The accurate identification of fruit flies is also an important part of the detection process. In the ERAfrica 'FRUIT FLY' project, attractants for fruit flies were tested in the field in South Africa and Côte D'ivoire. The potential of fruit volatiles for monitoring Afrotropical fruit fly pests was investigated in Reunion island. Field studies conducted in this project enabled testing and optimising the online multi-entry key for identification of African frugivorous tephritids developed by the Royal Museum for Central Africa, which also contributed to the molecular identification of problematic fruit fly vouchers through DNA barcoding.

Research studies on the ERAfrica 'FRUIT FLY' project have already made considerable progress in terms of developing effective detection tools for Afrotropical fruit fly pests. New information generated in this study will be packaged in the form of a fruit fly detection protocol which would be made freely available. Such information will be useful to growers,

agricultural inspectors and researchers and will help improve the management of quarantine fruit fly pests within fruit growing areas of Africa and the Indian Ocean region.

Abstract ID 105

Area-Wide Management of Chinese Citrus Fruit Fly in Tsirang, Bhutan Using Protein Bait Sprays and Orchard Hygiene

Kiran MAHAT[1], Phuntsho LODAY[1], Lhendup DORJI[1], Lakey LAKEY[2] and Graeme SANDERSON[3]

[1]National Plant Protection Centre, Ministry of Agriculture and Forests, Bhutan, Email: *kiranmahat@gmail.com*
[2]Horticulture Division, Ministry of Agriculture and Forests, Bhutan
[3]New South Wales Department of Primary Industries (NSWDPI), Australia

Background: The Chinese citrus fruit fly (CCF), *Bactrocera minax* (Enderlein), is one of the major fruit fly pests of mandarin (*Citrus reticulate* Blanco) in Bhutan. The recommended management strategies against this pest in Bhutan include a fortnightly protein bait spray application from early May to July and/or insecticidal cover sprays in conjunction with collection and destruction of dropped fruits. However, due to the lack of a coordinated approach in implementing these strategies, citrus growers fail to achieve the desired level of control, which in turn discourages its adoption by the farmers. Therefore, to showcase the effectiveness of a coordinated, community-based approach in managing the CCF, an area-wide fruit fly management programme was designed as part of a collaborative project between the Ministry of Agriculture and Forests of Bhutan and the Australian Centre for International Agriculture Research (ACIAR).

Methodology: The CCF management programme was implemented by the National Plant Protection Programme under the Ministry of Agriculture with field support from the district agriculture sector in a major mandarin growing area located in the south-western district of Tsirang, Bhutan. The trial site consisted of a contiguous area of approximately 120 hectares with a total of 270 farmers participating in the programme. Farmers were placed in groups, consisting of 5-10 farmers located within the same area, with a group leader in each group to monitor the programme. The programme was implemented in two consecutive seasons from 2013 to 2014.

Prior to implementing the programme, farmer's training on CCF biology and management was also conducted. As an incentive for participation in the programme, the inputs were made available to the farmers with funding support from the project. The field control programme involved a fortnightly spray of protein bait sprays (50 ml/ tree) from early May till the end of July and collecting and destruction of dropped fruits at a weekly interval that commenced from November to mid-December. Fruit fly damage assessment was undertaken in 10 orchards from both treated and untreated areas (where no fruit fly management programme was undertaken). In a single orchard 100 fruits were randomly sampled for fruit fly infestation.

Results: The results showed a significant difference in CCF infestation levels between the treated and the untreated sites in both the years. In the first year, 17.75% of the sampled fruits were infested in the treated sites compared to 58.75% in the untreated sites. In the second year, 19.25% of the sampled fruits were infested compared to 64.60% in the untreated site. In addition, an increase in yield and higher income were noted compared to years where no management practices were advocated.

Conclusion: The results show that the CCF can be effectively managed through a well-coordinated community-based approach and by employing environmentally-friendly management strategies like protein baiting and crop hygiene.

Abstract ID 116

F_1: Fast-Tracking *Eldana saccharina* Moths for Sterile Insect Release

Richard RUTHERFORD and Desmond CONLONG

SA Sugarcane Research Institute, South Africa, Email: *stuart.rutherford@sugar.org.za*

The African sugarcane borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is a serious pest. In the KwaZulu-Natal (KZN) province of South Africa an area-wide integrated pest management approach is promoted, which could include sterile insect releases (SIR). The efficacy of SIR depends on transfer of sperm carrying dominant lethal mutations from irradiated males to wild females. The ability of males to succeed in mating with wild females is most important, and this is particularly relevant when the mating system is complex, as in species such as *E. saccharina* where the males form leks, and where females are choosy. Because Lepidoptera are very radiation resistant, higher doses of radiation may result in less competitive male individuals. In addition, due to the stress and damage incurred during irradiation, handling, shipping, and release, SIR programmes usually produce males that are of lower quality than wild males.

Inherited sterility, or F_1 sterility, can be used to counter the negative effects associated with resistance to radiation. In this approach a radiation dose is determined that results in partial sterilization of male moths and as close to full sterilization of the more susceptible female moths as possible. Radiation-induced deleterious effects are inherited by the F_1 generation when released partially sterile males mate with wild females. Typically a male bias is then seen in a reduced F_1 field population, and both F_1 male and female offspring are more sterile than the irradiated parents.

Thus far, progress with *E. saccharina* SIR has relied on a remote irradiator located at Stellenbosch in the Western Cape province of South Africa. Transport of irradiated moths from this facility to KZN, which retain high enough quality for pilot-release studies, is currently not logistically feasible. In a modified approach we have proposed the mass-rearing and release of F_1 insects. To achieve this, separated male and female pupae are transported to Stellenbosch. Moths are irradiated, mated and eggs are returned to SASRI for mass-rearing.

In preliminary experiments male moths have been irradiated at doses ranging up to 200 Gy and mated with females in order to determine doses that optimize numbers of surviving larvae for mass-rearing, F_1 sterility and male bias. It is envisaged that the F_1 male will be of greater ‘fitness’ than its’ irradiated male parent, in part due to reduced handling, since there will be no irradiation step immediately prior to release. In addition a colony male bias should reduce the overall costs of rearing and release (female moths are approximately twice the mass of males).

Ultimately, this approach could see a single centralized irradiator and parental insect colony facility, serving a number of mass-rearing nodes located in the remote mill areas of the South African sugarcane industry.

Abstract ID 118

Field Application of Biocontrol Agents for Controlling the Red Palm Weevil

Mahmoud SALEH[1] and Gamal HEGAZY[2]

[1]Pests and Plant Protection Dept., National Research Centre, Dokki, Cairo, Egypt, Email: *mmesaleh@hotmail.com*

[2]Faculty of Agriculture, Plant Protection Dept., Ein Shams Univ. Shubra Elkheima, Cairo Egypt

Background: The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) is a destructive pest of palm trees of the genera *Phoenix* and *Cocos*. Infestations always result in the death of infested date palm or canary trees. Eggs are laid in wounded soft tissues or in leaf axils. Larvae excavate wide and deep tunnels into the trunks throughout their 2-3 months life-time. The developed pupae and even adults may be found with the larvae in the same infested site. Adults also aggregate in soil around the trunks as well as in the leaf petioles of palm trees. The cryptic feeding behaviour of the insect makes it difficult to control it with traditional insecticide applications.

Methods: Entomopathogenic nematodes (EPNs) and an entomopathogenic fungus (EPF) were applied for the biological control of the pest for the first time in Saudi Arabia in 2005. In Egypt, the EPNs were also applied in the field in 2010. Here we summarize methods and results of these applications.

Saudi Arabia applications: EPNs of the genera *Steinernema* and *Heterorhabditis* and the EPF *Beauveria bassiana* were sprayed alone and in combinations on RPW in leaf petioles and in soil around date palm trunks in three farms of 5 ha each. The nematode suspension was prepared at $4X10^4$ nematodes / L while the fungus suspension was prepared at $5X10^7$ spores / ml. Each tree received 5 L of either the nematode or the fungus suspension. Palm trees were cultivated at a density of 100 trees / ha. The population of RPW adults was monitored weekly in the treated and control farms throughout the experimental duration using pheromone traps. Trapped insects were inspected in the laboratory for nematode or fungus infection. Three applications, each of which consisted of three treatments either nematodes alone (N), fungus alone (F) nematode +fungus (N+F), were conducted in mid-November 2005, mid-February 2006 and mid-March 2006.

Egyptian applications: Water suspensions of four EPNs (*H. bacteriophora* HP88, *H. bacteriophora* S1, *H. indicus* and *S. abbasi*) were sprayed on leaf petioles and soil around trunks of date palm trees in a 5 ha date palm farm targeting the prepupae, the pupae in their cocoons and the adults of RPW.

Results: Saudi Arabian applications: The population of RPW adults dropped after each biological control application, but in different rates according the treatment. The combined

application gave the highest effect, followed by the nematode alone, and then the fungus alone (see the Figure).

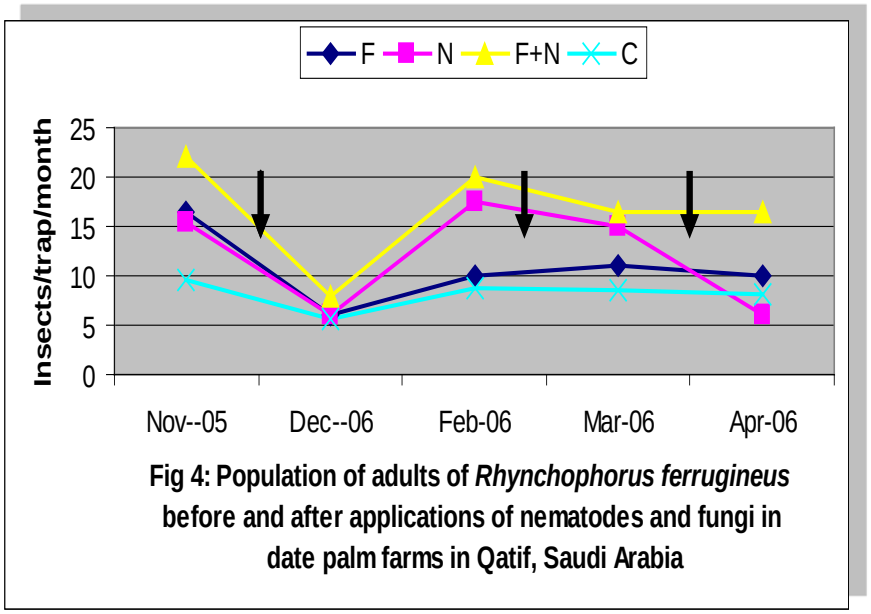


Fig 4: Population of adults of *Rhynchophorus ferrugineus* before and after applications of nematodes and fungi in date palm farms in Qatif, Saudi Arabia

Egyptian applications: Spraying EPNs on leaf petioles and soil around trunks of infested palm trees resulted in killing 50-100% of insects inside cocoons and 0-66.7% of adults in the soil. The most effective nematode was *H. bacteriophora*, while the least effective was *S. carpocapsae.*

Conclusion: The red palm weevil is a destructive pest to palm trees in many countries. Infestations frequently end with the death of infested trees. It is difficult to control the RPW because of its cryptic feeding inside tunnels in infested trees or the hiding of adults in the soil. Area-wide field applications of EPNs alone or in combination with EPF contributed to biological control of the pest since they were applied for the first time in 2005.

Abstract ID 126

Insights after Nine Years of Fruit Fly Monitoring in the San Francisco Valley, Brazil

Marijke N. DAAMEN, Fabrício A. DE SOUZA, Erivaldo A. DOS SANTOS, João L. DOS SANTOS and Jair F. VIRGINIO

Biofábrica Moscamed Brasil, Juazeiro-Bahia, Brasil, Email: *jair@moscamed.org.br*

The Mediterranean fruit fly (*Ceratitis capitata*) is a pest of great impact in the San Francisco Valley's orchards in Brazil and in particular it is a pest affecting exporters. This region produces about 90% of all Brazilian mangoes and more than 95% of all table grapes that are exported from Brazil. Besides that, this area is characterized by a semiarid climate (BSh according to Köppen-Geiger climate classification), presenting an average temperature of 24.8^{o}C and annual pluviosity of 435 mm. This climate combined with adequate irrigation and canopy management allows growers to harvest different fruit species during any time of the year independently of the season, giving them the opportunity to find the best trading periods in different international markets. *C. capitata* population dynamics is broadly studied under seasonal conditions and phenological development in other countries, but this specific condition of constant availability of hosts and favourable climatic conditions makes these observations unique.

Methodology: Currently there are 18,890 hectares of fruit crops being monitored for fruit fly species by Moscamed Brazil Facility under two programmes: *PAC* – Trapping and Control Programme and *PEC* – Pernambuco State Fruit fly Programme. Although both programmes aim to survey and evaluate fruit fly population dynamics, they have some different characteristics. PAC is a voluntary programme, which started in 2008. It is privately contracted by individual growers and mango/table grape exporters and is also used by APHIS/USDA for quarantine purposes and certification. Differently, PEC is a public financed programme which started in 2014 and is focused on smallholders who grow mangoes, table grapes, acerola and guava. PEC has surveillance and suppression purposes. PAC covers 5,590 hectares with 134 growers and 897 traps, while PEC covers 13,300 hectares with 2,560 growers and 1,330 traps. Both programmes were designed considering high density trapping in order to provide more detailed information regarding population dynamics in the San Francisco Valley and to support federal/state inspectors on suppression operations.

Results: Descriptive statistical analyses are showing that after 8 years of *C. capitata* monitoring (PAC) in the San Francisco Valley the average weekly FTD observed in mango orchards has been increasing year by year (in 2009 average flies/trap/day (FTD) was 2.13 while in 2016 it was 7.70). This scenario shows that suppression actions taken by growers are

not being sufficiently effective in the control of this pest, and that it is finding increasingly favourable conditions for development and establishment.

Data gathered in 2 years of PEC are also showing an increase in FTD and also confirm that other crops like acerola, guava and table grapes are also good hosts for Mediterranean fruit fly. Results also suggest that there is relation between FTD and fruit host availability.

Conclusion: In general, both monitoring programmes show that in the third quarter of each year there is an increase in the average weekly FTD; this period corresponds to the peak season of almost all fruit crops grown in this area.

Key words: control, Mediterranean fruit fly, monitoring, trap

Abstract ID 129

An Environmentally-friendly New Attractant (BIODELEAR) for the Mass-trapping of *Ceratitis capitata*

Nikos KOULOUSSIS

Aristotle University of Thessaloniki, Greece, Email: *nikoul@agro.auth.gr*

Background: The Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), is a serious pest of fruit crops and its control is based on several methods, including mass-trapping. For successful mass trapping, the attractant used does not only have to be highly efficient in capturing adults, but also be target- and sex-selective, produced at a low cost and be friendly to the environment and to the users. Here we present results of using the new female specific attractant BIODELEAR for the mass-trapping of *C. capitata* in citrus orchards in the Campos area of Chios. The results form part of a five-year LIFE programme aimed at developing an integrated strategy for the management of this serious pest.

Methodology: The study was conducted from September to December 2015. Mass-trapping was performed in four 0.5 ha plots using 50 plastic McPhail traps per plot baited with 17 gr of BIODELEAR. For comparative purposes we included three additional plots with traps baited with the "standard", commercially available attractant BioLure® (Suterra LLC, Bend, OR, USA). Likewise, we included four conventional plots treated with insecticides, as well as three organic plots receiving no treatments to serve as control. The efficacy of mass-trapping was evaluated by monitoring the level of *C. capitata* population using five separate McPhail traps baited with BioLure and five Jackson traps baited with trimedlure per plot. Infestation rates were also estimated by sampling and examining large numbers of fruits for oviposition stings and emerging pupae. Finally, to assess the environmental impact of each treatment, we monitored ground biodiversity of arthropods using pitfall traps.

Results and Conclusion: The results showed that mass-trapping with BIODELEAR resulted in a substantial reduction of the population of *C. capitata* compared to the organic control treatment. Although BioLure initially performed better, later in the season the two attractants converged in efficacy. In both the BIODELEAR and BioLure treatments sweet oranges and mandarin oranges were significantly less infested compared to the organic control treatment. Although BioLure appeared to be slightly better than BIODELEAR the two treatments did not differ statistically. Finally, in both the BIODELEAR and the BioLure treatments the diversity of ground arthropods was similar as in the organic control and significantly higher relative to the conventional insecticide treatment. These first results of the LIFE programme strongly suggest that mass-trapping with the new attractant BIODELEAR can effectively control the population of *C. capitata*, lower citrus fruit infestation and preserve biodiversity of arthropods. The results are particularly encouraging given the much lower cost and negligible toxicity of BIODELEAR compared to BioLure.

Abstract ID 130

Area-Wide Programme to Eradicate the European Grapevine Moth, *Lobesia botrana* in California, USA

Gregory SIMMONS[1], Matthew DAUGHERTY[2] and Lucia VARELA[3]

[1]United States Department of Agriculture, APHIS-PPQ-CPHST, Email: *gregory.s.simmons@aphis.usda.gov*
[2]University of California Riverside Entomology
[3]University of California Cooperative Extension

In the fall of 2009, the first confirmed North American detection of the European grapevine moth (EGVM), *Lobesia botrana*, was made in Napa County, California. Based on its status as a significant grape pest in other parts of the world, the establishment of EGVM in California presented significant production and export issues for grapes, as well as for other fresh market agricultural commodities.

Over the past seven years, an intensive California state-wide survey and area-wide eradication campaign was undertaken in partnership in cooperation with agricultural officials at local, State and Federal levels, university scientists and the wine, table grape and raisin industries. These efforts resulted in a dramatic decline in moth captures from over 100,000 moths in 2010, to one in all of 2014, and none in 2015 (Figure 1). In August of 2016, eradication was declared from all previously infested areas in California, USA.

The decision to pursue the eradication effort was based on the limited geographic area and host range of the EGVM infestation, the availability of several effective tools for monitoring and control, and the strong support of the affected grape production industry.

The eradication campaign employed a coordinated logistical, regulatory, and technical effort that included:
1) A state-wide monitoring effort using a network of moth pheromone traps and in field monitoring. These results were placed into a geographic information mapping system and were used to regularly communicate survey results to programme officials;
2) An area-wide application of hand-applied mating disruption dispensers to all infested grapes including their use in urban environments within infested zones;
3) Treatment coordinators implemented area-wide applications of insecticides with application timing determined by degree day modelling for each infested region;
4) A robust regulatory programme that initiated and maintained a quarantine of infested areas that regulated movement of fruit, farming equipment and winery processing wastes;
5) An extensive outreach programme to grape growers, wineries, pest control specialists and the public;

6) A technical advisory group was formed along with a robust methods development and research effort to provide guidance to the operational programme and to develop and test tools needed to support the programme.

An extensive methods development effort supported the programme and included developing enhanced detection methods for vineyards under mating disruption, testing efficacy and residual control of insecticides, testing mating disruption formulations, evaluating the impacts of winery processing methods on EGVM mortality, developing methods to determine EGVM biofix to improve degree day models in California, developing EGVM rearing methods, testing the quality of pheromone lures and trap monitoring; and a spatial analysis of trapping data to determine programme effectiveness and to analyse invasion pathways.

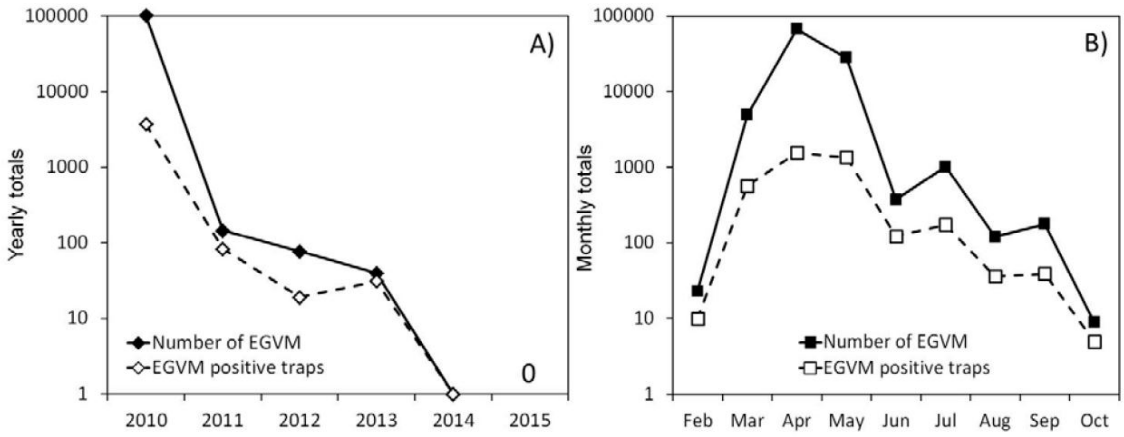


Figure 1. Overall A) yearly and B) seasonal trends (2010) in *Lobesia botrana* (EGVM) trap catch in California

Abstract ID 134

Attempt to Re-eradicate *Bactrocera dorsalis* (Hendel) from Mauritius

Preeaduth SOOKAR, Malini ALLECK, Indranee BULDAWOO, Sonia RAMJEE, Nazeer ALLYMAMOD, Fazilla KHAYRATTEE, Nausheen PATEL and Reena BHOYROO

Ministry of Agro Industry and Food Security, Agricultural Services, Entomology Division, Reduit, Republic of Mauritius. Email: *psookar@govmu.org*

Background: After two successful eradication campaigns of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) in Mauritius in 1996 and in 2014, the fly was trapped for the third time in October 2015 in a mango orchard in the West of the island.

Methodology: The protocol for eradication of *B. dorsalis* was immediately implemented: declaration of a quarantine area of 5 km radius around the detection site, placement of dry and wet traps, application of protein bait sprays, placement of MAT blocks, fruit stripping, fruit clean up, disposal of infested fruits, and collection and incubation of fruits and vegetables.

Results: Two months later, the fly was detected in the East of the island. Again the eradication measures were implemented. Modified waste brewery yeast mixed with an insecticide was used as protein bait sprays. Samples of vegetables, cultivated and wild fruits (1,800 samples for a total of 1,015 kg) were collected from the field and incubated in the laboratory from October 2015 to mid-December 2016. *B. dorsalis* was recovered from 24 samples of Indian almond, 21 samples of mango, 7 samples of guava, 5 samples of jew plum, 3 samples of avocado and 1 sample of jujube. During sanitation, 214 tons of fruits and vegetables were collected and buried. Dry traps baited with methyl eugenol and Malathion (1,068 traps) were placed over the island to monitor the fly population. *B. dorsalis* was detected in 72 traps in December 2016. The number of flies per trap per day ranged from 0.003 to 0.3.

Conclusion: After two mangoes and one Indian almond fruiting seasons, *B. dorsalis* did not spread over the whole island. The eradication measures implemented have contained the flies in the East and South-West regions.

Abstract ID 140

Area-wide Management with SIT of Mediterranean Fruit Fly in South Africa: New Management Techniques Showing Dividends

Jan Hendrik VENTER[1] and Charl BAARD[2]

[1]Deparment of Agriculture Forestry and Fisheries, Email: *byjhventer@gmail.com*
[2]Fruit Fly Africa

A mass-rearing facility to produce sterile male Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) started in 1999 in the Western Cape Province. The programme was initially underfunded and could only produce approximately 5 million flies per week. The number of flies was inadequate to reduce wild populations significantly in Hex River Valley in the Western Cape. The programme did show that with more funding, facilities can be improved, better quality control can be implemented and more flies can be produced. With occasional Government support, which allowed building a bigger facility, the introduction of a new genetic sexing strain and the implementation of a quality control system, more flies of better quality were produced per week, and more areas could be rolled out sporadically.

A new approach was implemented from 2009 with a memorandum of understanding (MoU) between The Department of Agriculture Forestry and Fisheries (DAFF) and the deciduous fruit and table grape industry. DAFF would provide 50% of the funds budgeted for, while the other 50% would be collected from growers through statutory levies. The MoU is renewed every three years.

In 2010 the new state of the art rearing facility was officially opened by the Deputy Minister of DAFF, which is now managed by a private company, *Fruit Fly Africa*, with board members representing growers from all the areas where area-wide management is implemented. Quality control was again improved, which included better larval diet, humidity control, egg harvesting and viability. Facility maintenance improved overall and a new Cobalt-60 irradiation source was installed.

Male fruit fly production increased to 25 million sterile males per week in 2015 and reached 56 million sterile males per week in 2016. Aerial releases were implemented and the area-wide approach also includes the use of improved monitoring systems, area-wide aerial baiting, ground bait application to eliminate hotspots, and an awareness and education programme.

The area-wide IPM programme now covers +/-30,000 hectares of commercial deciduous fruit and table grape orchards situated in 10 production regions. Of these hectares +/-15,000 hectares include SIT as part of their strategy. Aerial releases occur 35 weeks of the year over +/-38 000 hectares in these areas, and ground releases in hotspots are applied the other 17 weeks.

A further 5,000 hectares do ground releases year round. Average wild FTD's during the harvesting period for the larger areas in which SIT is applied as part of the programme has also decreased significantly during the past couple of seasons. The average FTD's during harvesting for the past 3 seasons were 47% lower compared to the 2011 data for the Hex River Valley and 76% lower for Ceres. For Elgin/Grabouw this decline was 23%.

South Africa is now aiming to manage some of the areas as areas of low pest prevalence.

Abstract ID 151

Preliminary Data on Distribution of Mediterranean Fruit Fly *Ceratitis capitata* (Wiedemann) in Albania

Anila GEZHILLI[1], Enkelejda DIKOLLI[2], Perparim KADRIAJ[2], Viola JANI[2], Eglantina ZAIMAJ[3], Silvia BINO[4] and Natasha HAKA-DURAJ[5]

[1]Faculty of Agriculture and Environment, Agricultural University of Tirana, Tirana, Albania, Email: *keladikolli@yahoo.com*
[2]Vector Control Unit, Institute of Public Health, Tirana, Albania
[3]Ministry of Agriculture, Rural Development and Water Administration, Tirana, Albania
[4]Institute of Public Health, Tirana, Albania
[5]Faculty of Agriculture and Environment, Agricultural University of Tirana, Tirana, Albania

A preliminary investigation on the geographical distribution of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) was conducted in different locations in Albania during the period of 2015-2016. Based on our data and historical ones observed over the last decades, it is one of the most destructive pests of different fruit crops.

The primary approach for monitoring of the pest consisted of advanced techniques and observations of different fruit trees including cherry, apple, fig, persimmon, plum, pear and citrus fruits. Furthermore, two different types of fruit fly traps, Jackson and Tephri, baited with 3 component female-biased attractants, were used during the last two years.

The monitoring of the pest was started by setting of the traps in both infested and intact fruit orchards at the beginning of spring. The traps were checked with two weekly frequency and number of flies and newly infested areas in each locality were recorded, separately.

According to our results, the higher index of *C. capitata*, with values from 4 to 9 flies per trap per day (FTD), was recorded in the case of citrus and persimmon fruits. Based on our data, there is difference of infestation varying with the seasons, where the higher value of *C. capitata* population was identified in Elbasan (FTD=4.8) in the fall of 2015, while in October the index ranged between 0.54 and 0.83. The most affected areas within Albania are Tirana, Vlora, Elbasani, Lushnja and Shkodra.

Key words: Albania, distribution, monitoring, Mediterranean fruit fly

Abstract ID 156

Integrated Management of *Bactrocera* Fruit Flies in Dragon Fruit Production in Viet Nam

Hien NGUYEN THI THANH, Thanh VU VAN, Trang VU THI THUY, Lien HA THI KIM and Khanh LE DUC

Plant Protection Research Institute, Viet Nam, Email: *thanhhien1456@gmail.com*

Background: Dragon fruit is one of the fruit crops in Viet Nam that is extensively being grown in the semi-arid area of Binh Thuan province and that plays an important role in the provincial economy. In order to control *Bactrocera* fruit flies, the Ministry of Agricultural and Rural Development has collaborated with FAO/IAEA to build up fruit fly low pest prevalence areas in the dragon fruit production province before integrating the Sterile Insect Technique to meet the government's strategy for domestic and international markets.

Methodology: The structure of the pilot area included 400 hectares of core zone and 600 hectares of buffer zone surrounding the core zone on all sides. Three suppression methods applied in the core zone consisted of (1) field sanitation, (2) male annihilation technique (MAT), and (3) bait spraying. Field sanitation included alternative host removal and destruction of unwanted dragon fruits. MAT was conducted by using methyl-eugenol and Fipronil blocks placed at 50 m interval across the fields, replaced every 10 weeks and maintained continuously throughout the year in the field. A mixture of ENTO-Pro 150 DD bait with Fipronil is applied weekly on the trees. Monitoring of fruit fly population was conducted in the core (12 traps), buffer (19 traps) and farmer's practice zones (5 traps) using a grid of traps baited with methyl-eugenol. The traps were inspected every 7-days and renewed every 6-weeks. Dragon fruit was sampled for fruit fly infestation for further evaluation of the effectiveness of the suppression measures.

Results: In 2016, the core zone and buffer zone reduced fruit fly population by 45.8% compared with the un-treatment area. The number of fruit fly per trap per day (FTD) was 1.8 and 2.2 in core zone and buffer zone, respectively, compared with 11.6 in untreated areas. However, the number of fruit fly caught from different traps in both core zone and buffer zone showed considerable variation. For example, in the buffer zone at the time of high pressure of fruit fly (early June), some traps caught less than 0.2 FTD, but others caught up to 33.3 FTD. The average percentage of dragon fruit damage in the core, buffer and farmer's practice zones were 1.4; 2.5 and 4.4%, respectively.

Conclusion: Area-wide suppression methods using MAT, sprays with protein bait and field sanitation were effective for controlling of *Bactrocera* fruit fly populations in dragon fruit farms in Binh Thuan province. However, the establishment and maintenance of an AWM programme requires knowledge about the alternative hosts, climate change, and grower education for continuing monitoring. *Key words:* Suppression, *Bactrocera*, dragon fruit

Abstract ID 158

Maximizing the Contribution of Biological Control to Area-wide Pest Management: Opportunities for Targeted Use of Nuclear Techniques

Kris WYCKHUYS

International Center for Tropical Agriculture (CIAT), Hanoi, Vietnam, Email: *k.wyckhuys@cgiar.org*

Pesticide use in Asia is surging. An unfortunate coincidence of successive pest invasions in several prime food or cash crops (e.g., cassava, coconut), a steady influx of cheap and generic insecticides, and the dismantling of some of the region's emblematic national IPM programmes have gradually pushed Asia's farmers back on the pesticide treadmill. Although mounting consumer awareness of food safety and environmental issues does generate lucrative opportunities for IPM and biological control, applied research in those domains remains limited and fragmented, particularly in Southeast Asia. Also, aside from its use in some Sterile Insect Technique (SIT) programmes for the integrated management of tephritid fruit flies in certain Asian countries, nuclear technologies have only been used to limited extent in pest management research, and especially in biological control.

We present a comprehensive overview of how nuclear techniques can help advance biological control research, and improve our understanding of on-farm biodiversity, trophic interactions in agricultural settings or broader impacts of human interventions on agro-ecosystem functioning. We describe how the analysis of carbon and nitrogen stable isotope ratios or the use of Rubidium sprays can reveal dispersal patterns of natural enemies within and between natural and agricultural habitats. We refer to new, ground-breaking work on the use of isotope markers to illuminate feeding behaviour of parasitic wasps under field conditions, or shed light upon below-ground drivers of pest control.

Lastly, we present evidence from European and North American studies on how nuclear techniques can guide the development of on-farm habitat manipulation tactics, e.g., the design of trap-cropping schemes or the deployment of beetle banks and flower strips. The above research advances are contrasted against the dearth of information on on-farm and landscape-level biological control processes in some of Asia's prime food, feed or cash crops.

We list a set of immediate opportunities, through which nuclear techniques can make substantial contributions to (area-wide) pest management research and pesticide-reduction efforts in the Asia region. The strategic use of nuclear techniques can provide Asia's biological control practitioners the necessary means to take full advantage of nature's services, bolster resilience of local agro-ecosystems in light of biotic and abiotic stressors, and safeguard the sustainability and long-term productivity of Asia's agricultural sector.

Abstract ID 165

Area-wide Management of Red Palm Weevil in Oman

Salim AL-KHATRI

Ministry of Agriculture and Fisheries, Muscat, Oman, Email: *salim_alkhatri@hotmail.com*

Background: Date palm (*Phoenix dactylifera* L.) plantations in Oman are infested by more than 54 arthropod species (insects and mites) affecting growth and yield of date palms quantitatively and qualitatively. Some of them attack fronds such as dubas bug (*Ommatissus lybicus* De Bergevin) and some attack fruits such as Lesser Date Moth (*Batrachedra amydraula* Meyer), whereas others attack the main trunk such as Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier).

The Red Palm Weevil (RPW) was first reported in the southern part of Oman in 1993. The available records since its introduction in 1993 showed that about 3,094 date palms were infested by the pest, of which 51.8% were destroyed due to heavy infestation in 1997.

Methodology: The implementation of Area-wild Integrated Pest Management (AW-IPM) concept for *R. ferrugineus* since 1998 was based on the implementation of quarantine laws and regulations to stop the introduction of any plant material from the plant family, Palmae into the country, and internal quarantine to stop its movement from the infested Wilayats to uninfected ones.

AW-IPM was also implemented through mass-trapping of RPW adults using aggregate pheromone baited traps for monitoring and controlling, extensive surveying to detect the infested date palms and chemical control by injecting insecticides into the infested date palms or destruction of the untreatable date palms.

Results: These efforts resulted in the destruction of 17.4% out of 41,192 infested date palms during 1999-2015. In addition, more than 555,812 adults of *R. ferrugineus* were trapped and removed from the infested area.

Conclusion: The concerted AW-IPM effort has resulted in making five of thirteen Wilayats infested by the pest free from RPW infestation by the middle of 2016.

Abstract ID 167

Insect Management in Wide Areas of Turkey

Yunus BAYRAM, Taha ASIKOGLU and Murat SAHIN

Ministry of Food Agriculture and Livestock, General Directorate of Food and Control, Ankara, Turkey, Email: *yunus.bayram@tarim.gov.tr*

Plant production is always one of the most strategic issues of the world and main component of human nutrition, therefore control of insects is an indispensable element in sustainability of plant production.

In Turkey, pest management is carried out according to the principles of Integrated Pest Management against 332 pests and the *Technical Instructions for Plant Protection* are an important source of information. Furthermore, the *Technical Instructions of Integrated Pest Management* are available in Turkey for 16 crops (apple, apricot, cherry, chickpea, citrus, cotton, hazelnut, lentil, maize, olive, peach, pistachio, potato, wheat, vineyards and greenhouse vegetables). In addition, special attention is given to the biological and biotechnical control, which is supported by subsidies since 2010, and 9.2 million TRY were given to producers in 2015.

Annual control programmes are implemented against sunn pest (*Eurygaster* spp. Hemiptera: Scutelleridae) and surveys were conducted by 550 teams in 56 provinces and 9,000 villages in 3.9 million ha and chemical control was applied in 410,572 ha by farmers in 2015. Also, 3.56 million parasitoids (*Trissolcus semistriatus* Nees) were reared and released for biological control of sunn pest in 2015. Grasshopper control is carried out during nymph period under State Control Support. In 56 provinces 34,754 ha were monitored and chemical control was applied in 16,430 ha in 2015.

In order to control *Ceratitis capitata*, a "Pilot Project on Mediterranean Fruit Fly" has been established for massive control of the pest in Adana, Hatay, Osmaniye and Mersin provinces. For surveillance of *Ceratitis capitata* throughout the country, the pest is monitored by 1,840 traps at 920 stations in 42 provinces within the "Project of Monitoring Mediterranean Fruit Fly". Against *Cydia pomonella* and *Lobesia botrana* Warning and Forecasting Systems (WFS) are in use and 301 WFS were used in 43 provinces at 262,551 ha in 2015.

In Turkey, a "*Pest Information System*" is in force for any pest introduced to the country. Annual survey programmes are under implementation throughout the country for 17 known and 11 unknown pests. In the "*Regulation of Plant Passport System and Registration of Operators*", the producers, traders and the ones storing plant and plant products and other substances which can carry the pests are registered and there is a follow-up on these materials. Turkish regulations on plant health are mostly in line with European Union regulations since Turkey is in the accession period to the European Union.

Abstract ID 178

The Monitoring and Management of the Chinese Citrus Fly, *Bactrocera minax* Based on Behavioural Research

Zhenzhong CHEN[1], Ping LI[2], Puyun YANG[2], Yongcheng DONG[1] and Changying NIU[1]

[1]College of Plant Science & Technology, Huazhong Agricultural University (HZAU), Wuhan, China, Email: *czzwell@163.com*

[2]Pest Control Division, National Agricultural Technology Extension and Service Center, Ministry of Agriculture, Beijing, China

Background: The Chinese citrus fly, *Bactrocera minax*, is an economically important pest of citrus in China, Bhutan, India, Vietnam and Nepal. It was removed from the national quarantine pest lists in China in 2009 owing to its wide-spread distribution across the country, and currently it is considered a major threat to the citrus industry in Asia. The frequent high infestation levels of *B. minax* in China emphasize the need for a more comprehensive understanding of its biology and behaviour so that efficient control measures can be developed and widely applied.

Methodology: In this study, we observed pupal development of *B. minax* to monitor the adult emergence in the wild. The ovarian development of the female flies was recorded in order to assess the sexual maturation of the flies. In addition, feeding, mating and oviposition behaviours were investigated during the occurrence of the adults in the field.

Results: Results revealed that pupal metamorphosis can be classified into 5 stages by which the emergence time can be predicted. Meanwhile, adult ovarian development was divided into 5 steps through which to estimate the peak time of oviposition. In the pre-oviposition period, the potential natural food substrates for adults include honeydew, nectar, bird faeces and sooty mould, which occurred mostly on non-host plants. After sexually maturation, adult flies shifted to citrus orchards for mating and oviposition. The mating pattern was described as follows: the male established its territory close to the citrus fruit, then the female landed on the fruit and perform ovipositor boring behaviour or oviposition, and finally the male mounted and copulated with the female.

Conclusion: Based on the above results, we concluded the sustainable management strategies for *B. minax* are as follows: 1. Protein baits as well as green fruit-mimic traps are implemented to attract and kill adults during the occurrence of adult flies from May to July; 2. In October specific bags are utilized to hold the infested citrus fruits for 7~10 days to kill the larvae inside. The strategies offered good management against *B. minax* over past several years in China.

Abstract ID 185

Scaling-up Biocontrol Using Sterile Insects as Phoretic Agents

Thierry BREVAULT, Thierry BALDET, Jeremy BOUYER and Helene DELATTE

French Agricultural Research Centre for International Development (CIRAD), Montpellier, France, Email: *delatte@cirad.fr*

Insect pests and vectors of plant and animal diseases have historically hampered agricultural productivity, negatively affecting food security and livelihood, particularly in less developed countries. The current global context - population growth, reduced natural resources and biodiversity, climate change, human mobility, and transboundary trade – further worsens these concerns and increases the risk of invasions and re-emergence of pests and vectors. The excessive reliance on broad-spectrum insecticides has shown strong limitations, including detrimental effects on both human and ecosystem health. Innovations relying on ecologically-based control are needed to respond efficiently to the global food demand, while addressing societal concerns for safer food, better health and environmental protection.

Biocontrol, which includes the use of living organisms (from micro- to macro-organisms), semiochemicals (e.g. pheromones) or natural substances (from mineral, plant or animal origin), is a promising way of significantly reducing the use of pesticides. The *Biophora* (Biocontrol Phoretic Agents) project proposed by CIRAD focuses on developing biocontrol systems based on phoresis (from the Greek phoros, "to carry"), a type of interaction between two organisms where an individual (phoronte) is transported by another (phoretic agent). The main objective is to boost the Sterile Insect Technique (SIT) by releasing sterile insects as conveyors of biocides for the control of conspecific or hetero-specific crop pests or disease vectors. Main targeted biological systems include fruit flies (Tephritidae), tsetse flies (Glossinidae), and mosquitoes (Culicidae).

Tackling such challenge requires a high-quality basic and applied research on phoretic agents (dispersion, mass-rearing, contamination, release systems, transmission), biocides (collection, screening, production, formulation, persistence, regulation, intellectual property), target systems (greenhouses, open fields, forests, herds, natural or urban areas), deployment scale (from local to large-scale), and risk assessment (non-target organisms, resistance evolution, environmental dissemination).

In parallel, an accurate evaluation of feasibility (cost-effectiveness), sustainability – including setting the basis for the assessment of long-term impact - and public acceptance of such technologies, is needed. Bringing together public and private stakeholders in the form of participatory platforms to elicit dialogue, partnership for intersectoral collaboration and decision-making, but also to inform civil society and public policy, is another major step.

By mobilizing a wide range of scientific skills to generate innovative and action-oriented knowledge, CIRAD is the key scientific partner for developing and scaling up operational pest and vector biocontrol within an integrated pest management perspective.

Abstract ID 189

Relative Abundance of Fruit Fly Species in One Agro-ecological Zone on Marula (*Sclerocarya birrea*) Trees in Botswana

Keabetswe NTLOGELANG and Patience MAWERE

Plant Protection Division, Ministry of Agriculture, Botswana, Email: *kntlogelang@gov.bw*

Background: Fruit flies are among the most destructive pests of important fruits and vegetables world-wide. However, very little is known about the diversity of fruit flies in Botswana. This study investigated fruit fly species diversity in one agro-ecological zone which was divided into three sites: Gabane, Mochudi and Rasesa in the Southern part of Botswana from December 2009 to March 2010.

Methodology: Chempac bucket traps baited with the parapheromones: cue-lure, methyl-eugenol, terpinyl-acetate and trimedlure and the food bait, BioLure were set up on malura trees (*Sclerocarya birrea*), to trap fruit fly adults and identify the species that occurred and determine their relative abundance in the three sites.

Results: The use of the different lures resulted in identification of fourteen species: 5 belonging to the genus *Ceratitis*; 6 in the genus *Dacus*; and one each in the genera *Coelotrypes*, *Isoconia* and *Trirhithium*. The most abundant species caught in the baited traps was *Ceratitis cosyra*. The study revealed greater fruit fly diversity than was reported from Botswana before, including species known to infest cultivated fruits of economic importance.

Conclusion: Future studies should include a wider area and be carried out over a longer period so as to establish the species which are prevalent in those areas. The information can be used to design fruit fly monitoring and management programmes that target fruit fly species found on known crop hosts in the area in order to minimize the fruit fly damage on fruits of economic importance and to promote international trade. Surveillance should also be done through fruit rearing to determine the host crops for some of the fruit fly species that were trapped during this study.

Key words: Marula, fruit flies, species diversity in Botswana

Abstract ID 196

National Fruit Fly Control and Eradication Programme in the Argentinean Northeast — PROCEM NEA

Diego QUIROGA, Wilda RAMIREZ and Cynthia RUIZ

National Service for Agrifood Health and Quality (SENASA), Buenos Aires, Argentina, Email: *procem@senasa.gob.ar*

Background: The National Fruit Fly Control and Eradication Programme of the Argentine Republic (PROCEM in Spanish) has been operating since 1994 for the control of *Ceratitis capitata* and *Anastrepha fraterculus*. It is based on a centralized coordination provided by the National Plant Protection Organization and regionalized implementation, with technical approaches adapted to the incidence of the pest in each fruit growing area.

In 2010, 78,715 hectares of the Argentinean Northeast Region were incorporated to the Programme, including 2,514 orange, tangerine and blueberry producers from the provinces of Corrientes and Entre Ríos, in order to reduce economic losses caused by the pest, estimated at 120,000 tons of citrus fruit over an average annual production of 700,000 tons.

Since this region is not geographically isolated and pest eradication is not considered feasible, the aim of the Programme in this region is to suppress the population levels, until they reach values compatible with the application of the Sterile Insect Technique.

Methodology: A detection system composed of 1,500 traps and fruit sampling was initiated to assess the presence, distribution and population dynamics of the pest, determining that the predominant species in the region is *C. capitata*, with isolated captures of *A. fraterculus*.

A change was proposed in the traditional modality of pest control, moving from individual farm control to an AW-IPM strategy that combines different phytosanitary procedures: aerial and ground insecticide bait application (Spinosad), field sanitation, and mass-trapping on the margins of urban areas. The organization of the producers was promoted in coordination with the NPPO and the governments of the two provinces involved. The strategy required the training of producers for the use of these new technologies.

Results: Between 2012 and 2016 the programme achieved the voluntary participation of over 1,300 producers in this control strategy, including their involvement in monthly aerial/ground insecticide sprays during the period of greatest incidence of the pest and weekly phytosanitary actions carried out by each producer. This method of control proved to be successful at the regional level, although throughout the successive years the strategy required a constant adjustment of different operational aspects. Such amendments consisted of scheduling treatments based on the frequent rainfalls, updating the planimetry of the surfaces to be treated, among others. The mean peaks of FTD (Flies/Trap/Day) in the core zone of the citrus growing area were reduced from 4 to 1.5 during the timeframe considered.

Conclusion: The strategy implemented represents an innovative way of addressing pest control in Argentina, where producers participate directly in area-wide application of phytosanitary procedures. To date, a large part of the region's fruit producers are organized and involved in the control of the pest. This implied reversing the lack of unity and differences that were observed between producers before the start of the Programme. A challenge for the next years is to ensure the active involvement of all fruit producers in the region and to perform pilot tests for the integration of the Sterile Insect Technique.

Abstract ID 197

Mexican Plant Health, the Key to the World Fruit Market: Fruit Flies Case

Francisco Javier TRUJILLO-ARRIAGA, Francisco RAMIREZ-Y-RAMIREZ, Guillermo SANTIAGO-MARTINEZ, Ruben Angel HERNANDEZ-LIVERA, Arturo BELLO-RIVERA and Fabian SANCHEZ-GALICIA

Dirección General de Sanidad Vegetal del SENASICA, Ministry of Agriculture, México City, Mexico, Email: *francisco.ramirez@senasica.gob.mx*

Introduction: The "*Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria*" is Mexico's administrative organism responsible to protect the agricultural, aquacultural and livestock resources from pests and diseases of quarantine and economic significance. Strategic priorities among others are: to control the main insect pests, such as fruit flies, and to support the fruit exports. The National Fruit Flies Programme (NFFP) has the objective to detect, control and prevent the dispersion of fruit flies populations (*Anastrepha ludens*, *A. obliqua*, *A. striata* and *A. serpentina*) and to avoid the introduction and establishment of Mediterranean fruit fly *C. capitata* and other exotic fruit flies into the national territory.

Methodology: The NFFP is operated under an area-wide integrated pest management approach, including, as a main axis, the sterile insect technique and biological control. All work is done within a national legal frame for the operation of the NFFP, the regulation of movement of fruit fly hosts and the implementation of National Emergency Protocol.

Results: From 1992 up to date 1´001,409 km^2 (51.11% of the national territory) has been recognized as free of fruit flies of the *Anastrepha* genus and 97,406.6 Km^2 (10.07% of national territory) as a low prevalence area of these pest insects. Most importantly, 100% of the national territory is free of the Mediterranean fruit fly, with benefits to more than 1.8 million hectares cultivated with 15 vegetable and fruit host commodities: avocado, coffee, pumpkin, green pepper, peach guava, mango, apple, melon, orange, papaya, cucumber, water melon, tomato, and grape, which represents a production volume of 19.2 million tons and an approximated value of 97,000 million Mexican pesos.

As a result, during 2016, 315,000 tons of mango were exported to 19 countries; 99,000 tons of sweet citrus to 12 countries; 9,500 tons to the United States. Mexico is ranked first in the world exporting commodities subjected to irradiation as a phytosanitary treatment, with 12,300 tons shipped a year. For the NFFP, economic assessments indicate a benefit/cost ratio of 22/1 for mango and 19/1 for sweet citrus. For the Moscamed Programme this rate was 112/1.

Currently, a new Mediterranean fruit fly mass-rearing facility is being built in Metapa de Domínguez, Chiapas, with an investment of 800 million pesos and a production capacity of 1,000 million sterile pupae per week and irradiation capacity based on Cobalt-60. The facility will be the most modern in its genre worldwide, incorporating the use of technology for the

optimization of the processes of mass-rearing insects and is built following all rules to preserve the environment.

Conclusion: Phytosanitary programmes have contributed to Mexico being the world's 12th largest food producer, with agrifood exports growing at an average annual 5.4% in the last 3 years, earning 30 billion US dollars in foreign exchange this year.

It should be noted that agrifood exports remain above the sale of oil and worker remittances, thereby confirming the importance of phytosanitary investment in the world’s horticultural trade.

Abstract ID 202

Effect of Pre-release Raspberry Ketone Supplements on Ability of Queensland Fruit Fly to Tolerate Desiccation and Nutritional Deprivation

Humayra AKTER, Polychronis REMPOULAKIS and Phillip W. TAYLOR

Macquarie University, Sydney, Australia, Email: *humayra_05@hotmail.com*

Background: Queensland fruit fly (*Bactrocera tryoni*) males exhibit substantially accelerated sexual maturation when their pre-release diet is supplemented with raspberry ketone (RK) during the first two days following emergence. Accordingly, RK shows promise as a pre-release supplement for sterile insect technique (SIT) that might increase the proportion of released sterile flies that mature in the field and the period over which released flies are active in mating with pest populations. However, metabolic enhancers such as RK might also diminish the ability of flies to tolerate harsh environmental conditions following release in the field.

Methods: Flies were fed a diet of sugar and yeast hydrolysate (3:1) that contained 0%, 1.25% or 5% RK for 48 hours after emergence. To assess effects of deprivation under normal controlled environment conditions (25^{o}C, 65% RH), groups of 30 male or female flies were maintained in 5 litre cages that contained (1) 3:1 mix of sugar and yeast plus water, (2) water only, or (3) no food or water. To assess effects of deprivation under desiccating environment conditions without food or water, flies were maintained individually in sealed glass vials that contained silica gel.

Results: Under normal controlled environment conditions, females lived longer than males, flies receiving both food and water lived longer than those receiving water only, which lived longer than those receiving neither food nor water. Flies receiving low doses of RK lived longer than those that received either high doses of RK or none. Under desiccating environmental conditions, females lived longer than males, and both males and females lived longer when provided no RK than when provided either dose of RK.

Conclusion: RK shows potential as a pre-release supplement for *Bactrocera tryoni* as part of SIT releases, accelerating development and increasing mating propensity of young flies. Under non-desiccating conditions, RK supplements do not diminish survival and at low doses may even increase survival. However, under extreme desiccating conditions, RK supplements do diminish survival. The potential implications of these findings for operational SIT conditions warrant further investigation in order to identify conditions under which RK supplements might be beneficial or detrimental to SIT efficacy.

Key words: Bactrocera tryoni, sterile insect technique, metabolic enhancer, desiccation

Abstract ID 210

The Occurrence of Fruit Flies (*Rhagoletis batava*) and Damage Caused by Them on Sea Buckthorn Production of Mongolia

Undarmaa DAVAASAMBUU[1] and Sainzaya TSEGMED[2]

[1]Mongolian University of Life Sciences, Ulaanbaatar, Mongolia, Email: *undarmaad@hotmail.com*
[2]Western Branch Research Institute, PARI, MULS

Background: Common sea buckthorn (*Hippophae rhamnoides*) is a spiny deciduous shrub with fruits having a high vitamin C content exceeding that of lemons and oranges. It is native to sandy habitats across Eurasia and resistant to wind, heat and frost.

The sea buckthorn fruit fly, *Rhagoletis batava* Hering (see Figure 1), is considered to be the most dangerous pest in sea buckthorn production of Mongolia. It has spread through most sea buckthorn plantations and infested either wild or cultivated berries grown in Uvs, Khovd and Zavkhan provinces. Due to fruit fly infestation farmers had a yield loss of 60-70% yearly in those areas.

Methodology: During this investigation yellow traps have been used to detect fruit flies, to determine the flight periods and the abundance of adults in these periods. Traps were placed between 1st week of May and 3rd week of September during 2008-2014. In our experimental field we randomly selected 20 plots where the traps were deployed and checked each trap every 5 days.

This information was used to determine the first occurrence, abundance and mating of the fruit flies.

Results: According to our investigation the occurrence of *R. batava* flies started from 3rd week of June (the pupal stages had overwintered on the soil surface). High abundance was observed during the 2nd and 3rd week of July and during this period they also mated. After that, the flight intensity of the fruit flies slowly decreased, which indicates the starting of oviposition.

We assumed climate effects on the occurrence of the sea buckthorn fruit fly (see Graph 1). The flight period started when the air temperature was above 18^0C and increased at 22-23^0C. Our results show that the air and soil surface temperature correlated strongly ($R^2 = 0.93$ and $R^2 = 0.94$) to both, the occurrence and the abundance of the fruit flies, but the precipitation didn't show an effect ($R^2 = 0.32$).

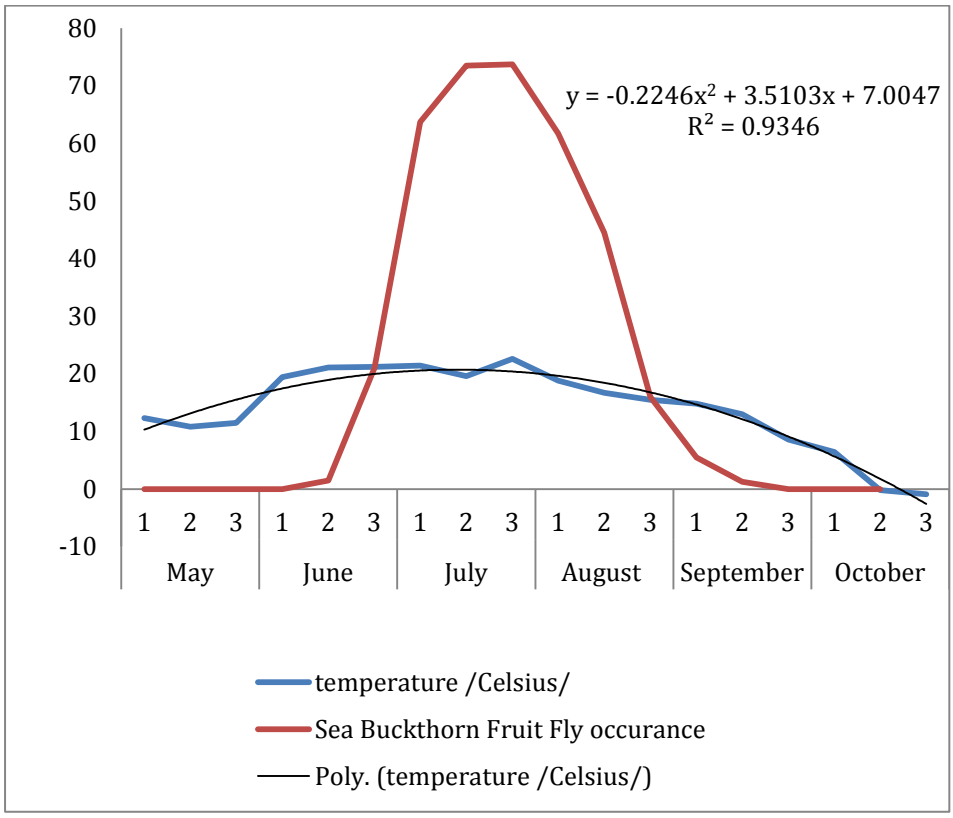
Conclusion: 1. The most suitable period for controlling adults by yellow traps lies between the 2nd and 3rd week of July and for the pupal stage in early spring and late fall.

2. The oviposition period falls between the end of July and the end of August when the berries are setting on sea buckthorn. Larvae emerge 7-10 days later and cause damage to the

berries. When the harvesting starts in September, berries are rotten and mature larvae will fall down to the ground, turning into the pupal stage at which they overwinter.

Fig. 1. *Fruit fly (*Rhagoletis batava*) and its damage on sea buckthorn berries.*

Table 1. *Sea buckthorn fruit fly (*Rhagoletis batava*) population in Uvs province area (2008-2014).*



Abstract ID 213

Area-wide Ecological Engineering for Rice Pest Management in China

Zeng-rong ZHU, Ping QIAN and Hongye LI

Zhejiang University, Zhejiang University, Hangzhou, China, Email: *zrzhu@zju.edu.cn*

Background: Within the crop ecosystems, there are plentiful innate factors regulating the dynamics of pest herbivore populations. An ecological engineering (EE) approach is a design of sustainable ecosystems that integrates human society with its natural environment for the win-win benefit for both.

Field spiders' assemblages were testified as the key regulators of planthopper population dynamics in subtropical mountainous rice fields. However, in subtropical and temperate agricultural plains, these innate factors, as ecosystem services, have been normally underestimated due to the heavy use of harmful pesticides. The negative relationship of the small brown planthopper, *Nilaparvata lugens* (Stål), density to landscape diversity in northern Zhejiang plain fields in early summer indicated the potential for area-wide ecological engineering for rice pest management.

Methodology: A series of field experiments with the objectives of augmentation of local natural enemies before the early season of the main crop were carried out in the plain paddy. A summarized protocol called, shortly in five continuous letters, LMNOP, representing respectively *Landscape designing, Monitoring the target pests, Natural enemy conservation and enhancing, Optimization of sowing and transplanting dates of mid-resistant crop variety and nutritional management, and Pesticide application in need-based*.

This package of technical components aims to increase the regulating capability of natural enemies through the functions of NATURE, i.e. *Nutrition including nectar and pollen, Alternative preys and hosts, Tunnel for movement, Umbrella (hoUse, refUgia) sites for REproducting of long-term flowering, early season growing plants*. Field demonstration and a package of multiple technical communication tools were used to deliver the EE to farmers for area-wide up-scaling.

Results: The population growth of the main herbivores in the demo pilot fields was decreased and the peak densities of the main damage generation shifted to lower ones, below the economic injury levels. The unit grain yield was not lower than in conventional farms, but economic and ecological gains were higher.

LMNOP has been transmitted to agricultural extension workers and larger and small scale farmers. Very positive feedback from audiences, paper media, and electronic media readers suggest multiple media communication outlets are excellent tools for biodiversity education and outreach.

Conclusion: Landscape design-based area-wide ecological engineering for rice pest management in subtropical and temperate plains is implementable and successful.

Abstract ID 228

Lessons Learned from AW-IPM Control of Fruit Flies to Combat Cassava Pink Mealybug in Thailand

Watchreeporn ORANKANOK[1], Areepun UPANISAKORN[1] and Kris WYCKHUYS[2]

[1]Ministry of Agriculture and Cooperatives, Bangkok, Thailand, Email: *watchreeporn@yahoo.com*
[2]International Center for Tropical Agriculture (CIAT) Hanoi, Vietnam

Background: During 2008-2010 the exotic pest cassava pink mealybug (CPM), *Phenacoccus manihoti*, rapidly spread and infested many provinces of Thailand, causing severe losses to overall cassava plantations, especially in 49 out of 77 provinces. The infested areas comprised approximately 1.60 million hectares. Millions of poor cassava farmers lost their incomes and their livelihoods were threatened. In order to combat CPM, Thailand identified area-wide pest management strategies and approaches for mealybug management by applying gained experiences from the cooperative work with FAO/IAEA in area-wide integrated fruit flies control using sterile insect techniques and other methods.

Methods: A surveillance system for CPM was established and conducted nationwide, which confirmed that the highly invasive CPM causes serious losses to cassava production. Therefore, a square grid was deployed and integrated pest control methods were designed as appropriate approach. *Anagyrus lopezi* was introduced into Thailand from Benin in 2009 for biological control of CPM, while the local predatory lacewing, *Plesiochrysa ramburi*, was mass-reared and released weekly. Field sanitation before planting was strongly recommended, followed with chemical control for pre-planting treatment of cutting stalks.

One of the most important factors to bring success was the good cooperation from cassava growers under the strategy of farmer empowerment through the community pest control centres (CPCC), which was carried out by farmers' group.

Various organizations and stakeholders which included DOAE, DOA, KU and TTDI joined hands to conduct a training programme, with the assistance and support from relevant international organizations, such as FAO and CIAT. The training aimed at enhancing better understanding and knowledge of CPM control by farmers so that they could set social order in their own communities to control the movement of contaminated stalk.

Since 2011-2013, more than 40 million pairs of *A. lopezi* and 50 million green lacewings *P. ramburi* were produced and released. Three years of exerted efforts to combat CPM by adopting area-wide CPM management show that CPM infested areas decreased from around 300,000 hectares in May 2009 to 10,88 hectares in October 2013. Since 2013 up to present, the number of infested areas has remained under 1,000 hectares, thereby reducing the risk of losses and area-wide insect pest management strategy was proved to be a sustainable control of CPM.

Abstract ID 229

Comparative Efficacy of Integrated Pest Management Methods for the Suppression of *Bactrocera dorsalis* (Diptera: Tephritidae) on Citrus

Vincent UMEH, Daniel ONUKWU and Mercy OLANIYI

National Horticultural Research Institute, Nigeria, Email: *vumeha@yahoo.com*

Background: The advent of the Oriental fruit fly *Bactrocera dorsalis* in Nigeria tremendously affected the fruit industry and thus hampered the bourgeoning domestic and export fruit trades. To remedy the situation and restore producers' confidence for a sustainable fruit production, various control methods were tested and released for on-farm validation. The present study seeks to test the efficacy of some IPM methods for the suppression of *B. dorsalis* in farmers' orchards in Kaduna state, Nigeria.

Methodology: Two hectare portions, each of five citrus orchards measuring 12 - 15 ha in area, were selected in part of Kaduna state in the Guinea savanna agro-ecological zone of Northwest Nigeria. The efficacy of the selected IPM methods was tested over three citrus fruiting/harvest seasons of October 2015 - January 2016, May - August 2016, and October 2016 - January 2017; the three seasons were used as replications for the treatments.

Different control or IPM methods were assigned to each of the orchards as follows: (i) Bucket trap baited with methyl-eugenol (ME) + DDVP insecticide, (ii) Bucket trap baited Timaye® granules (methyl-eugenol + deltamethrin), (iii) Bucket trap baited with methyl-eugenol + DDVP and bottle trap baited with brewery yeast waste (BYW), (iv) Bucket trap baited with Timaye® granules and bottle trap baited with BYW, (v) two spot sprays of BYW + DDVP. Temperature (^{o}C) and Relative Humidity (RH) records were taken during population assessments for the three fruiting/harvest seasons.

Results: There were no significant differences between the populations of fruit flies in the baited bucket traps, either singly or in setups where bottle traps baited with BYW were present. However, the IPM setups whereby the combination included bottle traps with BYW caught significantly more fruit flies than those with only bucket traps. Significantly lower numbers of citrus fruits were attacked in IPM combinations whereby bucket traps with the parapheromes + insecticides were set up together with bottled traps with BYW + DDVP. *B dorsalis* populations increased with increasing temperature and RH. *B. dorsalis* populations decreased during the dry season with lower temperatures and RH.

Conclusion: The results showed that better control was achieved by combining the parapheromone traps, which are attractive to *B. dorsalis* males, with protein bait traps, which are mainly attractive to the females. The implications of choice of baits, appropriate combinations and their arrangement are also discussed.

Abstract ID 233

Towards the Creation of a Pest Free Area for Fruit Flies (Diptera: Tephritidae) in the Mashonaland Central Province of Zimbabwe

Jeremiah MASOKA[1,2], Louisa Delfin Mutsa MAKUMBE[1,3], Nhamo MUDADA[1], Christabel NYANGA[1,4], Cames MGUNI[1] and Godfrey CHIKWENHERE[1]

[1]National Plant Protection Organisation of Zimbabwe, Harare, Zimbabwe, Email: *jerrymasoka@gmail.com / masokajerry05@gmail.com*
[2]Faculty of Natural Resources and Agriculture, Great Zimbabwe University, Masvingo, Zimbabwe
[3]Department of Zoology and Entomology, University of Pretoria, Republic of South Africa
[4]Faculty of Agriculture, Midlands State University, Gweru, Zimbabwe

The presence of the Oriental fruit fly, *Bactrocera dorsalis* in the Mashonaland Central Province of Zimbabwe was reported in September 2012 by the National Plant Protection Organisation of Zimbabwe (NPPOZw). Mashonaland Central Province is located in the northern part of the country and borders with the Republic of Mozambique to the north and the Republic of Zambia to the north-west. Since the detection of this quarantine pest, management practices were immediately instigated. The measures focused mainly on management in fruit and vegetable production areas, service centres, border entry/exit points and major road transnets, so as to curb further spread of the pest to the rest of the country. The province produces huge volumes of fruit to supply the local market as well as for local consumption. The fruit and vegetables include citrus, mango, guava, tomato and banana.

The measures put in place included the use of Male Annihilation Technique (MAT) using INVADER-b-Lok blocks, M3 Bait Stations, intensive trapping using methyl-eugenol baited traps, orchard sanitation, use of entomopathogens (*Metarhizium anisopliae*), and spraying fruit orchards with GF-120 Spinosad and Malathion 25% W.P. INVADER-b-Lok blocks were deployed at a density of three blocks per hectare in fruit and vegetable production areas and two in non-production areas, three M3 bait stations were put in fruit and vegetable production areas and at least one McPhail type trap was placed per hectare. For orchard sanitation, augmentoriums were deployed across the provinces with more emphasis on fruit and vegetable production areas. The augmentoriums are being used to conserve natural enemies, while at the same time trapping all the flies within fallen fruits. A pilot suppression site was also established using entomopathogens (*Metarhizium anisopliae*). There fungi are put inside Lynfield traps at a density of three traps per hectare with a food based attractant. Aerial sprays with Malathion 25% W.P. and GF120 Spinosad were also applied fortnightly during the fruiting season and once every month during non-fruiting season.

Since the beginning of the project in 2013 there has been a sharp decline of fruit flies, from an average during peak fruit production of 23 flies/trap/day (FTD) to 0.008 during the non-fruiting season and 2.67 during the fruiting season.

Abstract ID 234

Monitoring Activities on Invasive Fruit Flies of Economic Importance in Austria

Alois EGARTNER, Christa LETHMAYER, Richard GOTTSBERGER and Sylvia BLUEMEL

Austrian Agency for Health and Food Safety (AGES), Institute for Sustainable Plant Production, Email: *alois.egartner@ages.at*

Background: Fruit flies (Diptera: Tephritidae) are among the most important pests of orchard crops worldwide. From the up to 200 species that are considered as pests, the most important example for Austria is the European cherry fruit fly (*Rhagoletis cerasi*), which is also the most important pest in Austrian cherry production.

Compared to countries with more favourable climatic conditions for the development of fruit flies, the number of harmful species recognized in Austria is low. However, increasing numbers of interceptions of tephritids at the entry points of the European Union, and more findings of non-native fruit flies in Austria in previous monitoring activities, indicate an increasing relevance of these pests for the Austrian fruit production.

Host plants for some of the most destructive invasive tephritid species are available and grown to a considerable extent in Austria. While the climatic conditions in Austria might possibly limit the potential establishment of non-native fruit fly species originating from warmer climatic areas, there is still a risk of establishment in warmer regions of the country especially in the context of climate change.

Methodology: Consequently, a national monitoring for relevant fruit fly species, focusing on species from the genera *Ceratitis* and *Bactrocera*, was established in Austria in 2016. In total 68 traps with male-specific lures were employed to attract and catch the adult flies. The traps were placed within the range of relevant host plants, mainly peach or apricot, on 17 sampling sites in six Austrian federal provinces mainly in the eastern part of the country.

The monitoring was carried out in a two-week interval from mid-June until the end of November 2016. Identification of the caught fruit flies was performed on morphological basis and confirmed with molecular diagnostic methods for single flies.

Results: Monitoring activities resulted in a total of 766 caught fruit flies from the genus *Ceratitis* and two fruit flies from the genus *Bactrocera*. Detailed results of the monitoring will be presented.

Abstract ID 243

Application of Geographic Information Systems in the Pest Management in Wide Areas

Alejandro MOLERO and Mariel VANIN

ISCAMEN - Instituto de Sanidad y Calidad Agropecuaria de Mendoza, Mendoza, Argentina, Email: *presidencia@iscamen.com.ar*

The Agricultural Sanitary and Quality Institute of Mendoza "ISCAMen" carries out insect pest suppression and eradication projects (*Ceratitis capitata*, *Cydia pomonella*, *Lobesia botrana*) in the Province of Mendoza, Argentina, working on a total of 278.514 has (plus urban areas), using the area-wide IPM concept. This project has as its main objective to minimize action times for control through the use of an integrated geographic information system. The development of the system mentioned will be possible through the integration of next components Spatial Databases Georeferenced Monitoring System “IDE” Data Infrastructure Online Control Panel.

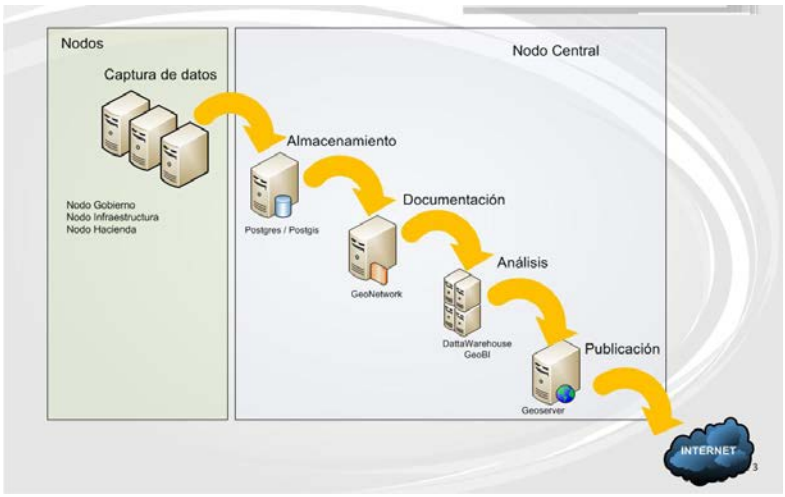
Spatial Databases: It is a centralized, open source Postgres / Postgis spatial database management system. The information is stored in a database saving the coordinates of the traps and random sampling performed in the area. A software allows online data publication, with industry standard WMS and WFS formats through a GeoServer service. It has metadata services with its Geonetwork module.

Georeferenced monitoring system: The synchronized systematization of monitoring data collection is done with fullcrum.app, transferring data/images to a centralized database. All information collected is referenced with GPS, allowing the viewing in a map of the work in real time. The system allows:

• To create custom forms for field data collection
• To capture geospatial information; Photos, videos, audios, bar codes and information defined in the forms
• The use of base maps: Google, Open Street Map, or custom maps
• To display of captured data quality controls and analysis in a web-map interface; to edit and modify staff assignments; to query data using search and filtering tools
• To use the activity view to view real-time data collection status
• To keep the history of modifications and updates in any device and a retrospective analysis
• To play the spatial content of the video by browser, to see where the video clips were collected in a map interface
• To adjust automatically forms and data on real time
• To organize work for mobile users for assignment of tasks and processing of work orders
• To configure activities within the organization from mobile computers or the Web.

Spatial Data Infrastructure "IDE": It is built with a geoportal, allowing interoperability of spatial and non-spatial data between its organisms, using catalogues and standards with free and open source software The IDE will allow searching, locating, viewing, downloading or requesting different types of geographic information related to the territory of Mendoza and integrating through the Internet the data, metadata, services and geographic information produced in Mendoza. The IDE can be part of a network of spatial data infrastructures with nodes at the regional level. Users can be different organisms and free users.

Online Control Panel: It will allow visualizing the main key indicators of each pest in real time and will serve as a basis for decision making in management. This module will be connected to the central database and will display its indicators as the information is updated permanently. The representations will be maps, diagrams, tables, graphs.


Nodos
Captura de datos
Nodo Gobierno
Nodo Infraestructura
Nodo Hacienda
Nodo Central
Almacenamiento
Postgres / Postgis
Documentación
GeoNetwork
Análisis
DattaWarehouse
GeoBI
Publicación
Geoserver
INTERNET


Resumen de Periodos


Periodo Anterior
Comparativo entre periodos

Abstract ID 256

Control of Fruit Flies in Peru

Jorge Antonio MANRIQUE LINARES and Carlos RIVERA-TEJADA

SENASA, Ministry of Agriculture, Peru, Email: *jmanrique@senasa.gob.pe*

Background: In Peru, the species *Ceratitis capitata* was recorded for the first time in Huánuco in 1956, while the genus *Anastrepha*, native to the American continent, is present with 76 species.

Methodology: The National Fruit Fly Surveillance network consists of 36,000 Multilure and Jackson traps, with food and sex attractants respectively, installed in the field using GIS technology, in 80- or 20-hectare quadrants. The installation density increases according to the objective (prospecting, suppression or eradication).

The surveillance of exotic flies is done with the installation of a specific trapping network located in the main points of entry to the country such as ports, airports, border complexes and places of tourist importance.

Results: With this surveillance system installed in Peru, it has been determined that the complexity of species of fruit flies increases from south to north and from the coast to the jungle. In addition, the data generated are entered into a computer platform that facilitates access to and preparation of information for analysis and decision-making.

SENASA has implemented three projects for the eradication of pest fruit flies, including actions such as chemical control (ground spraying of toxic bait), cultural control, and ground release of sterile flies in urban areas or difficult to reach places, campaigns of awareness and communication, quarantine protection and legal control.

The intervention area during 2016 was approximately 800 thousand hectares of agricultural land benefiting 301,223 producers, owners of 170 thousand hectares with horticultural production. The eradication projects are based on the implementation of four stages: Prospecting and monitoring, Suppression, Eradication and Prevention, and the duration and intensity of actions vary according to the progress achieved in the control of the target pest.

Conclusion: At present, two southern departments have been recognized as Pest Free Areas and 11 departments are at low prevalence levels, achieving an annual saving of USD 400 per hectare, and avoiding a loss of approximately USD 15 million at the national level per year.

Abstract ID 259

Utilization of *Fevillea cordifolia* as Trap Plant to Control *Anastrepha grandis* ((Macquart, 1846) in Panama

Pablo RODRIGUEZ

National Directorate of Plant Protection, Ministry of Agricultural Development of Panama, Panama City, Panama, Email: *prodriguezg@hotmail.com*

Background: Anastrepha grandis is a highly restricting pest to the international trade of fruit of the Cucurbitaceae family. Adults of this species were detected in Panama in 2009. This detection led to the application of phytosanitary measures for the containment of the pest.

From 2009-2012 the applied phytosanitary measures applied were: legal control, phytosanitary surveillance, suppression with selective insecticides and intensive sampling of fruits of cultivated and wild cucurbitaceae.

Methods: In 2012 larvae of *A. grandis* were detected in fruits of the wild cucurbitacous *Fevillea cordifolia* L. In 2014 a control campaign was started based on search, location and registration of sites with the presence of *F. cordifolia*, where systematic applications of GF-120-NF-Naturalyte-02% ® were carried out, as well as permanent installation of bait stations with Cera Trap® and cultural control.

Results: The control measures applied in *F. cordifolia*, under the concept of a trap plant, are considered to be effective, since the level of *A. grandis* catches in the traps of the official phytosanitary surveillance system has decreased significantly.

During 2016 and so far in 2017 no catches of the pest have been reported. However, in bait stations with CeraTrap®, these catches persist, although they have decreased compared to previous years. In Panama *F. cordifolia* is the only host found for *A. grandis*. Its use as a trap plant has allowed suppressing the populations of the pest to levels not detectable by the official monitoring traps.

Conclusion: The results obtained support the current negotiation with USDA-APHIS to modify the internal quarantines, and increase the production opportunities of the farmers of the infested zones. This experience is applicable to other countries with the presence of *A. grandis* and *F. cordifolia*, as to suppress populations, mitigate the risk of dispersion of the pest and reduce the severity of restrictive measures imposed by commercial partners.

Key words: Cucurbitaceae, Anastrepha grandis, Fevillea cordifolia, *plant trap, suppression*

Abstract ID 260

National Fruit Flies Detection System (NFFDS) in Chile: 50 Years of One Successful Plan

Ricardo RODRÍGUEZ P., Carlos LOBOS A., Ruth CASTRO F., Jocelyn YEVENES F., Alejandro BARRA P. and Pedro JARA R.

Agriculture and Livestock Service (SAG), Plant Protection Division, Santiago-Región Metropolitana, Chile, Email: *ricardo.rodriguez@sag.gob.cl*

Background: The first detection of a Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) in Chile occurred in 1963 in the north of the country. The National Fruit Flies Detection System (NFFDS) was created in 1980 as the *Project for* Eradication *and Exclusion of Fruit Flies in Chile*. It has been used as a guiding and unifying plan for the different actions to combat the fruit fly outbreaks that from time to time occur in different regions of the country. The project was approved as a state entity and its implementation was delegated to SAG, the Chilean Ministry of Agriculture and Livestock.

Chilean fruit production represents the No. 1 item in agricultural and forestry exports, with an amount of USD 4,000 million in return. Generally speaking for every dollar invested in the Fruit Flies Programme in Chile, about USD 1,000 return to the country.

Methodology: The NFFDS protects 300,000 hectares of fruit production and its base is the permanent surveillance for non-native fruit flies from the genera *Ceratitis, Anastrepha and Bactrocera* through a network of 15,500 traps. In 1992, in Arica, the Sterile Insect Production Center was built with a maximum production capacity of 50 million irradiated males per week; these insects are preventively released in the same city close to the border with Peru; they have also been used in case of Mediterranean fruit fly outbreaks in the central region.

The NFFDS success is based on four fundamental pillars:
1) Strict quarantine control at each point of entry into the country,
2) A permanent surveillance system throughout the country,
3) Contingency plans to respond quickly and effectively to any detection of non-native fruit flies in the country, and
4) Signature of agreements with neighbouring countries on matters related to fruit flies.

Results: Incursion verification and elimination has been effective for Mediterranean fruit fly and a very occasional foray *of Bactrocera dorsalis* in Easter Island (2011) and a single detection of *Anastrepha fraterculus* in the city of Iquique.

Conclusion: Thanks to the NFFDS and its four working pillars, tending to the early detection and eradication of incursions in the shortest possible time, it has been possible to effectively maintain Chile as a free country for fruit flies of economic importance, for more than 50 years.

Abstract ID 262

National Fruit Flies Plan in Colombia

Emilio ARÉVALO, Ángela CASTRO, Blanca VARGAS, Maribel HURTADO, Pedro RODRÍGUEZ, Zaida FLOREZ and Moisés GORDILLO

Dirección Técnica de Epidemiología y Vigilancia Fitosanitaria, Instituto Colombiano Agropecuario, Bogotá, Colombia, Email: *emilio.arevalo@ica.gov.co*

Background: Fruit flies, especially of the *Anastrepha* genus, are widely distributed in Colombia. Meantime, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), was detected for the first time in 1986; nevertheless, since then its distribution within the country has been restricted to certain atreas. However, over the last years the Mediterranean fruit fly has been detected in areas where it had not been previously been recorded, affecting different commercial fruit crops.

Methodoloy: The Instituto Colombiano Agropecuario (ICA), through the Technical Direction of Epidemiology and Surveillance of Plant Health, executes the National Fruit Flies Plan (NFFP) that contributes to detect non-native flies, determine the population distribution and control fruit flies important for the Colombian fruit production.

The ICA, since 1998, has implemented the national fruit fly surveillance for Colombia. Currently, it covers 194,980 hectares in 26 departments through 132 surveillance networks for the genera *Anastrepha, Ceratitis* and *Bactrocera*, following the FAO/IAEA parameters using Jackson and McPhail traps and lures such as hydrolysed protein, trimedlure, methyl-eugenol and cue-lure.

Results: Since 2008, five areas have been established as areas of low pest prevalence and two as pest free areas for fruit fly species. One of them is the basis to access the United States market for golden berry (*Physalis peruviana* L.) without the need of a quarantine treatment.

Conclusion: The actions for the NFFP have permitted to determine the distribution of different fruit fly species in Colombia and to detect non-native and regulated pests opportunely. This has allowed taking informed decisions for the control programme of the Mediterranean fruit fly in different areas and also maintaining low pest prevalence and pest free areas for other fruit fly species.

Key words: Fruit fly, surveillance, pest, low pest prevalence, pest free areas

Abstract ID 265

Sterile Insect Technique against Pink Bollworm in Pakistan

Waqas WAKIL

Department of Entomology, University of Agriculture, Faisalabad, Pakistan, Email: *waqaswakeel@hotmail.com*

Cotton is the main cash crop of Pakistan and makes our country the fifth largest producer of cotton, the third largest exporter of raw cotton and of cotton yarn in the world. The crop covers about 15% of the cultivable area, contributes about 10% to GDP and 55% to the foreign exchange earnings of the country.

The cotton crop is attacked by many insect pests, but pink bollworm (PBW) (*Pectinophora gossypiella* Saunders) is the worst enemy that not only causes major losses to the crop but also affects the lint quality. Crop losses may range from 20-30% depending upon the level of infestation and weather conditions. In Pakistan, after inflicting a loss of Rs 125 billion in 2015, the dreaded pest re-emerged in 2016 as a major threat to the cotton crop and led to a 21% reduction in total grown crop for the season. In 2015-16, the total value of the cotton crop was roughly $33 billion Rs (corresponding to a 28% decline in crop yield), the lowest in past eleven years leading to 0.5% reduction in national economic growth. The main reason behind this huge loss is the breakdown of resistance of 1st and 2nd generation *Bt*-cotton to PBW. The pest was already resistant to all conventional insecticides that are applied almost 10-17 times per season.

One of the most promising alternative control strategies of PBW is the area-wide integrated use of the sterile insect technique (SIT). It had been successfully tested against this notorious pest using a pilot sterile male release programme and radiation-induced F_1 sterility in Pakistan. The literature shows that work on evaluating radiation effects on males, pupae or F_1 progeny of PBW initiated in late 1980's. Research organizations like Nuclear Institute for Agriculture and Biology, Faisalabad (NIAB) and Nuclear Institute of Agriculture, Tandojam (NIA) are mainly working on this technique and provide sterile insects for further investigations in laboratory or field studies.

Conclusion: Considering the importance and usefulness of this technology, likewise in other countries, there is need to resume the research and exploitation of irradiated PBW as an effective component of area wide-integrated pest management (AW-IPM) of this insect pest in Pakistan.

Key words: SIT, *Pectinophora gossypiella*, area-wide management, cotton, Pakistan

Abstract ID 269

The Cactus Moth (*Cactoblastis cactorum*) Eradication Programme in México

Arturo BELLO-RIVERA[1], Francisco Javier TRUJILLO-ARRIAGA[1], Rebeca GUTIERREZ[2], Rui PEREIRA[3] and Walther ENKERLIN[3]

[1]Dirección General de Sanidad Vegetal del SENASICA, Ministry of Agriculture, México City, Mexico, Email: *arturo.bello@senasica.gob.mx*
[2]Department of Entomology, Michigan State University, East Lansing, Michigan, USA
[3]Insect Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria

Introduction: The spectacular success of using the cactus moth (*Cactoblastis cactorum* Berg) in the control of invasive, alien opuntias has been cited often in biological control literature. This insect is highly damaging to plants of the Opuntia family, and is considered one of the most successful biological control agents in the world. For example, in Australia it eliminated approximately 25 million hectares of *Opuntia* spp. where they had become major weeds. In Mexico, *Opuntia* spp. are important components of natural ecosystems and also have many commercial uses.

The detection network of the preventive programme against cactus moth detected two outbreaks. The first one on 10 August 2006, in Isla Mujeres, municipality of Isla Mujeres, Quintana Roo and second on 4 May 2007, in Isla Contoy, Municipality of Isla Mujeres, Quintana Roo, where immediate eradication activities were implemented through the National Service for Health, Safety and Food Quality (SENASICA).

Methodology: The strategy used in the cactus moth eradication programme in México was based on an area-wide integrated pest management (AW-IPM) approach. The operation of a highly sensitive trapping system allowed the early detection of the pest outbreaks, which made it possible to respond immediately so as not to increase the extent of the affected areas. The following actions were immediately implemented: Removal and control of hosts (*Opuntia dillenii* and *Nopalea cochenillifera*) in natural and in housing areas; exchange of *Opuntia* plants by ornamental plants; utilization of plants as sentinels and pheromone traps to monitor and control the population; use of selective insecticides, especially in areas with very dense plant populations and difficult access, and the release of sterile moths (sterile insect technique).

For the implementation of the eradication actions, the FAO/IAEA, the United States Department of Agriculture (USDA), the North American Plant Protection Organization (NAPPO) and the Mexican Government provided technical and economic support. This was used for research including development of pheromone, traps, artificial rearing, radiation

biology, dispersal, ecological and economical studies, and for surveys, field infrastructure, suppression activities, technology transfer, outreach and training.

Results: The eradication of two outbreaks of cactus moth from Quintana Roo State was achieved in 2009, maintaining the country as free of this pest. The following formal Public Notices were published in the *Official Gazette of the Mexican Federation*:

- On 26 March 2009, Notice by which the outbreak of cactus moth (*Cactoblastis cactorum* Berg.) was declared eradicated from Isla Mujeres, Municipality of Isla Mujeres, Quintana Roo.
- On 12 October 2009, Notice by which the outbreak of cactus moth (*Cactoblastis cactorum* Berg.) was declared eradicated from Isla Contoy, Municipality of Isla Mujeres, State of Quintana Roo.

With the eradication of the cactus moth from these sites in Mexico, 250,000 hectares of cultivated *Opuntia* were protected, as well as its diverse uses including: fruit and green vegetable consumption, animal fodder, medicinal products, cosmetics, pigment and fencing. Added to these are the extensive wild areas of cactus, a vital part of biodiversity of the arid ecosystem in the country, estimated at over 3 million hectares.

Conclusion: The cactus moth programme in Mexico is an example of successful eradication of an invasive species. The AW-IPM approach was able to combine technical, economic, human and political resources to achieve the objective. Nevertheless, the threat prevails in view of the pest's presence in the Caribbean and the south-eastern USA. The Mexican government under the ongoing Programme of Phytosanitary Epidemiological Surveillance is prepared for the timely detection and response to a new cactus moth incursion.

Abstract ID 275

Expanding Area-wide Mediterranean Fruit Fly Management in Morocco through the Implementation of a Mass-rearing Facility

Atifa QADDA, Aziza EL YAEGOUBI and Jamila WADJINNY

Sanitary and Food Safety National Office, ONSSA, Morocco, Email: *atifa.qadda@gmail.com*

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), attacks a large number of fruit species, including citrus and other cultivated and wild hosts. It is considered one of the most economically damaging pests of citrus production in Morocco. In addition, Mediterranean fruit fly infestation in Morocco causes major problems to exports due to quarantine restrictions. It limits market access and raises the risk of losing existing markets. The existence of a great number of attacked host plants makes Mediterranean fruit fly control difficult and costly. Therefore, the strategy against Mediterranean fruit fly must be part of an area-wide management programme that integrated the use of less polluting area-wide alternatives including the Sterile Insect Technique (SIT).

In Morocco, since 2006, SIT has been implemented in a limited area in Souss valley (4,500 ha of citrus). Sterile male pupae were initially imported from Portugal and then, more recently, from Spain. However, the high cost of this operation, in addition to limited scale and other factors, have prompted government officials and the citrus industry to think of ways to build and operate their own mass-rearing and sterilization facility. The main objective of this national project is to eventually expand SIT application to all citrus production areas in the country (120,000 ha).

During the first SIT phase the mass-rearing facility, which will be built in Agadir and has already been designed, will allow to cover the overall citrus production area of Souss Valley (40,000 ha of citrus) and additional buffer areas to prevent re-entry of fertile females from surrounding wild host areas. The estimated budget for the construction is up to US $5 million, which will be shared through a public-private scheme between the Moroccan Government and MAROC-CITRUS, with technical support from the International Atomic Energy Agency (IAEA).

The construction of the mass-rearing facility is planned to initiate in May 2017 and it will be completed by October of 2018. The ultimate goal is to produce 200 million sterile flies per week.

Abstract ID 276

Area-wide Integrated Control of Fruit Flies in Thailand

Suksom CHINVINIJKUL[1], Werrawan SUKAMNOUYPORN[1], Wanitch LIMOPASSMANEE[2], Alongkot UTHAITANAKIT[3] and Watchreeporn ORANKANOK[4]

[1]Irrradiation for Pest Management Section, Department of Agricultural Extension, Ministry of Agriculture and Cooperatives, Chatuchak, Bangkok Thailand 10900, Email: *chinvinijkuls@gmail.com*
[2]Thailand Institute of Nuclear Technology (Public Organization)**,** Nakhon Nayok Thailand
[3]Chanthaburi Provincial Agricultural Extension Office, Chanthaburi Thailand
[4]Department of Agricultural Extension, Bangkok Thailand

Background: Bactrocera dorsalis (Hendel) and *B. correcta* (Bezzi) are classified as economically important pests that cause major losses and constrain the international fruit trade of Thailand. With International Atomic Energy Agency (IAEA) support, Thailand conducted a programme integrating the sterile insect technique (SIT) in fruit flies population suppression.

Methods: Since 1991, in collaboration with the Office of Atoms for Peace, irradiated pupae were weekly shipped and directly ground released in 720 ha of mango orchards in Ratchaburi province. In 1995, a small pilot mass rearing facility with capacity to produce 40 million pupae per week was established. Area-wide IPM approach was accepted since 2001 and SIT was implemented in suppression strategy to control *B. dorsalis* and *B. correcta*. Since 2003 sterile fly releases of both species were expanded from the first 720 ha area to 3,440 ha and a second area covered 3,670 ha of mango plantation in Phichit province. The white-striped strain of *B. dorsalis* was developed by Thailand Institute of Nuclear Technology (TINT) in 2007 and sterile flies were released weekly over 2,590 ha of tropical fruit plantation in Chanthaburi province. Appropriate control methods and technologies were transferred and integrated in each area depending on community participatory decisions.

Results: Fruit damage was reduced from over 80% to an average less than 3.6% in the last five years (2000 to 2004) in Ratchaburi, meanwhile it was reduced from 43% in 2002 to 15% in first two years in Phichit. Exportation possibility were opened for mango produced in these areas to some of the most stringent and lucrative markets. For Chanthaburi province, average sterile to wild ratios were increased to 6.39, 8.04, 3.32, and 7.30, while flies/trap/day (F/T/D) were maintained at 0.76, 0.58, 0.73, and 0.75 in 2013, 2014, 2015 and 2016 compared with 1.20 and 2.37, respectively, in 2012.

Conclusion: Sterile insect releases integrated with other environmentally-friendly control techniques can reduce fruit damage from *B. dorsalis* and *B. correcta* and maintain the wild population of *B. dorsalis* at a low level, while increasing sterile to wild ratios in the treated areas. Further efforts following the International Standards for Phytosanitary Measures could

lead the active area to reach the goal of fruit fly low prevalence under National Plant Protection Organization certification. Currently, the Chanthaburi area is under IAEA technical cooperation programme to enhance agricultural productivity by supporting the production of fruit-fly free commodities and meeting international standards. Furthermore, IAEA has supported a number of IAEA fellows from Asia-Pacific countries to be trained in fruit fly mass rearing and other related activities in Thailand.

Key words: *Bactocera dorsalis*, population suppression, area-wide IPM, white-thoraxed strain

Abstract ID 288

Pilot Study on Suppression of Melon Fly, *Zeugodacus cucurbitae*, in Seychelles

Samuel BRUTUS[1], Bob PETROUSSE[1], D. LESPERANCE[1], L. CADEAU[1], M. NICOLAS[1], M. NOURICE[2], R. GOUVINDIN[2], Stuart SMITH[3], Preeaduth SOOKAR[4], Rui PEREIRA[5] and Randy STRAVENS[1]

[1]National Plant Protection, Seychelles Agricultural Agency, Ministry of Fisheries and Agriculture, Victoria, Mahe, Seychelles, Email: *brutussamuel@gmail.com*

[2]Vegetable Research Station, Seychelles Agricultural Agency, Ministry of Fisheries and Agriculture, Anse Boileau, Mahe, Seychelles

[3]Department of Primary Industry and Fisheries, Darwin, Northern Territory, Australia

[4]Entomology Division, Ministry of Agro Industry and Food Security, Reduit, Mauritius

[5]Insect Pest Control, Joint FAO/IAEA Division on Nuclear Application for Food and Agriculture, Vienna, Austria

Background: Melon fly, *Zeugodacus cucurbitae* (known previously as *Bactrocera cucurbitae*), was introduced into Seychelles in 1999 and has become a major pest on cucurbits on the main islands of the Seychelles archipelago. After the introduction, the Seychelles government initiated a campaign to eradicate melon fly in the country. However, due to technical and economic reasons the objective was changed to suppression.

Methodology: With this objective, a pilot suppression study was conducted for 3 months (the cucumber crop cycle) on Mahe Island during 2014. The study area was about 8 km^2 in extent, and the following suppression methods were integrated: (1) sanitation; (2) male annihilation technique (MAT) blocks baited with cue-lure (CUE); (3) bait spay with GF-120 Spinosad; and (4) mass-trapping with GF-120. To evaluate the impact of the suppression, a network of 41 Tephri-traps with CUE as attractant was installed and fruit sampling for larval infestation was conducted before and during the pilot trial.

Results: Adult populations captured in CUE traps decreased from about 6 flies/trap/day (FTD) to <1 FTD, and fruit infestation decreased from about 90% at commencement to 20% during the trial. Farmers' incomes increased due to an increase in marketable cucurbits. These promising results showed the importance of integrating several fruit fly management methods, and emphasized the advantages of an area-wide approach to population control rather than the field-by-field approach.

Conclusion: A follow-up study to combine the above suppression methods with the sterile insect technique (SIT) is planned for initiation as soon as sterile melon flies become available.

Key words: *Zeugodacus cucurbitae*, sanitation, male annihilation technique, bait sprays, bait stations, adult surveillance, larval surveillance.

Abstract ID 292

Exploring the Biocontrol Agents for Ricania Simulans (Walker, 1851) and its Microbiota

İkbal Agah İNCE and Ömer Faruk TASTAN

Acibadem University, School of Medicine, Atasehir, Istanbul, Turkey

Recently, tea farms, fruit plantations and other important crops in the Black Sea region of Turkey have been under the invasion of a Hemipteran polyphagus pest insect, Ricania simulans. The population is threatening the area-wide agricultural production in the region since 2006 and the distribution of the pest is expanding to neighboring regions.

Interestingly, this pest invades tea gardens. Turkey is the one of the middle-scale tea producer countries and tea production is one of the important agricultural activities of the region. R. simulans has been known in Georgia for 60 years, but only recently in the Black Sea region of Turkey. Very often polyphagus pest insects move to another plant species, and can even feed on herbs, due to the shortage of food after consumption of one type of agricultural plant. However, it is little known for tea pests and their biological control agents, as the tea plant has very few known pest insects.

In the region, to prevent losses, owners of tea gardens, vineyards or agricultural lands use chemical formulations for plant protection against pest insects. Due to the many negative side effects of chemical pesticides on human and environmental health, attention must be paid to the use of environmentally safe, biological control options against this insect. Therefore, search, detection and isolation of biological agents from the population of R. simulans is being carried out to promote the use of biological control agents as natural enemies of this pest.

In our study, we designed experimental procedures to unravel the microbiota of the pest. 16S and 18S rRNA analysis of the microbiome of the pest was carried out to identify the endosymbionts and if possible any entomopathogenic microorganisms. Exploring the microbiome of this unknown pest will facilitate development of mitigation strategies using microbial biopesticides or developing area-wide management strategies.

By performing the current project, the most effective biological control agent could be proposed to be mass-reared for field control of the pest. Secondly, the identification of the microbiota of the pest will bring an opportunity to develop another commercialization path for producing incompatible insects for area-wide control of the pest population. For the incompatible insect technique (IIT) perspective, there could be an increasing interest in developing and applying the sterile insect technique (SIT) and the incompatible insect technique (IIT), separately or in combination. SIT is an environmentally-friendly and species-specific method of pest control based on the release of large numbers of sterilized insects.

Competition for mating between wild and sterile males results in a decrease in the number of fertile matings and a decline in the overall population size. SIT has been successfully implemented against various pest insect species, including several Tephritidae.

Key words: Ricania simulans, *Microbiota, Symbiont, Entomopathogen*

Poster Presentations Session 2: Mosquitoes and Human Health

Abstract ID 6

Role of Silver Nanoparticles in Mosquitoes Control

Farkhanda MANZOOR

Lahore College for University, Department of Zoology, Lahore, Pakistan, Email: *doc_farkhanda@yahoo.com*

Background: Mosquitoes transmit major diseases and major control is only by chemicals, but there is dire need to develop new environmentally-safe yet effective methods. A major bulk of effort in terms of newer methodology has mainly been concentrated on synthesis aspects. During the present study silver nanoparticle's (AgNPs) role against mosquitoes control was checked.

Methodology: Silver nanoparticles were prepared from aqueous leaf extract of the plant *Azadirachta indica* (Neem). Their effect on mosquito vectors, i.e. *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* was assessed in the laboratory. The synthesized AgNPs were characterized by using UV-visible spectroscopy and X-ray diffraction (XRD) techniques. Toxicity assays for silver nanoparticles were carried out at room temperature (25±3 °C) as per WHO protocol (WHO 1982, 1985). A homogeneous stock solution from silver nanoparticles was prepared (100 mg/L).

Results: The results showed that AgNPs have maximum absorption at 430 nm with mostly spherical particles and face-centered cubic (fcc) crystalline structure. The mosquito larvae (1st, 2nd, 3rd and 4th instars) were exposed to a specific concentration of AgNPs, i.e. 0.5 mg/L for 24 hours, 48 hours, 72 hours, 96 hours and 120 hours. Larvicidal activity of AgNPs with LT50 and LT90 value were 34.84 and 70.64, 46.43 and 160.01, and 45.58 and 112.26 for *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus* respectively.

The reason behind the effectiveness of the nanoparticles against mosquitoes is the mechanism by which they work. These particles were very minute and they got inserted into DNA of the mosquitoes and destabilized their machinery of making biochemicals like lipids, carbohydrates, protein, etc. The mosquitoes became unable to digest and metabolize lipids and carbohydrates remain undigested due to which their level of glycogen increases.

Conclusion: Azadirachta indica is a potential biolarvicidal agent for mosquito control with no hazardous effects on non-target organisms.

Abstract ID 9

Mosquito Surveillance and Control: Challenges, Management and Opportunities in Sovereign Base Areas (SBA) in Cyprus and Overseas Territories

Angeliki-Kelly MARTINOU

Joint Services Health Unit British Forces Cyprus, Cyprus, Email: *af.martinou@gmail.com*

During the last two decades, nearly a third of all recorded events related to emerging infectious diseases are vector-borne. Amongst these, mosquito transmitted pathogens are of prime concern. Recently the Zika virus outbreak in the Americas prompted world-wide concerns stressing the role of surveillance as a key component of any local integrated vector management programme. Quantifying human risks by determining local vector presence and abundance is essential.

Surveillance of putative vectors such as native and invasive mosquitoes that can lead to a successful targeted management programme is not a simple task as it requires a great deal of interdisciplinary collaboration under the 'One Health' concept. Efficient risk assessment (RA) depends on high-quality data on the presence, abundance and distribution of mosquito vector species and in-depth knowledge of their ecology.

The current and previous efforts of an active surveillance scheme for native and invasive mosquitoes run by the Joint Services Health Unit in the Sovereign Base Areas (SBAs) in Cyprus will be presented. Integrated mosquito management approaches as well as challenges faced will be discussed. Opportunities for research and collaborations will be highlighted.

Abstract ID 15

Towards the Implementation of the SIT in Argentina in Integrated Pest Management to Control *Aedes aegypti*, Vector Mosquito of Dengue, Zika and Chikungunya

Eulogia KAIRIYAMA[1], Jorge DESCHUTTER[2], Ricardo OLMEDO[3], Rosa Del Carmen PIRAGINE[4], Marianela GARCIA ALBA[1] and Fabián ZELAYA[5]

[1]Aplicaciones y Tecnología de las Radiaciones, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina, Email: *kairiyam@cae.cnea.gov.ar*
[2]Comité Ejecutivo de Desarrollo e Innovación Tecnológica, Ministerio de Educación, Cultura, Ciencia y Tecnología de Misiones, Posadas, Misiones, Argentina
[3]Dirección de Saneamiento Ambiental, Ministerio de Salud de Misiones, Argentina
[4]Secretaría de Salud Municipal de Posadas, Misiones, Argentina
[5]Dirección Control de Vectores del Municipio de Posadas, Misiones, Argentina

Background: Since the reintroduction of the mosquito *Aedes aegypti* to Brazil in 1975, all the Southern Cone countries have been reinfested with the vector. Reinfestation in Argentina was detected in 1986 in the provinces of Misiones and Formosa, which both share borders with Brazil and Paraguay. At present *Ae. aegypti* mosquitoes have spread to most provinces of the country, extending up to the 40° South Latitude.

Dengue occurs annually, associated with the summer-autumn period. The control of the vector is carried out in coordinated actions among municipality, province and nation, and mosquito control is directed towards elimination of breeding areas, actions to prevent mosquito bites, and fogging during outbreaks.

The combined use of the Sterile Insect Technique (SIT) and Incompatible Insect Technique (IIT) may offer a safe and efficient complement to decrease the incidence of diseases transmitted by infected mosquitoes. Argentina has started a National Project on the feasibility of integrating this technique into the control measures for *Ae. aegypti*, the vector of dengue, chikungunya and Zika. This project receives technical support from the IAEA through the projects RLA5074, INT5155 and ARG0015.

Methodology: At laboratory scale, at Ezeiza Atomic Centre (CAE) pre-release experiments have been carried out to gain experience in rearing methodology, male pupae sterilization procedures and quality control of irradiated male mosquitoes. Eggs of *Ae. aegypti* were collected in Resistencia-Chaco (27,5°S; 59°W) (strain Res-1); they were reared at the CAE laboratory and the sterilization experiments were done with the 3rd generation, using a Gamma Multipurpose Irradiation Facility at a dose rate of 10 Gy/min and the absorbed doses were determined by Fricke. The samples of male pupae, group of 50 units each, were irradiated at incremental doses from 0 Gy to 100 Gy. Post irradiation quality control (emergence, fitness, longevity) of irradiated pupae were analysed for each irradiation dose

and compared to non-irradiated controls. Another strain of *Ae.aegypti* was collected in Posadas-Misiones (27,4°S; 56°W) (strain Pos-1).

Results: High quality colonies of *Ae. aegypti* Res-1 (6 generations) and Pos-1 (3 generations) are developing. Preliminary results indicate that the minimum sterilization dose for males of *Ae. aegypti* Res-1 was 70 Gy; further experiments using the strain Pos-1 are on-going.

Semi-field release experiments are planned for early 2017 and will be completed at CAE. Santa Rita (Posadas city) district was selected as a pilot site, and collection of baseline data has now started in this field.

Up-scaling of the rearing of *Ae. aegypti* will be done in the Centre for Development and Technological Innovation in Posadas, together with the Environmental Sanitation of Misiones province government and the Laboratory of Vector Control, Health Ministry of Posadas Municipality. A pilot release study of sterile mosquitoes in a neighbourhood of Posadas city is planned for 2018.

Abstract ID 18

Assessment on Radiation-induced Sterility and Longevity of *Aedes aegypti* (Diptera: Culicidae) Strain of Swat, Pakistan

Inamullah KHAN, Junaid AHMAD, Gul Zamin KHAN and Alam ALAMZEB

Pakistan Atomic Energy Commission, Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Pakistan, Email: *inamullah17@gmail.com*

Background: Vector-borne diseases are emerging threats in Pakistan and thus require particular attention. The status of dengue cases is increasing each year. Pakistan has experienced seven mild and two severe dengue outbreaks during the last 25 years. The last two severe dengue outbreaks resulted in 21,685 confirmed cases and 363 deaths during 2011 throughout the country and during 2013, six thousand dengue confirmed cases and 57 fatalities were recorded from the Swat district in Khyber Pukhton Khwa (KP) province.

Methodology: In our efforts to combat the menace of dengue we have initiated both short-term management strategies and long-term vector suppression focusing on the integration of the Sterile Insect Technique (SIT) for dengue control.

In this context, sterilization of male *Ae. aegypti* pupa was conducted with radiation doses of 0,30, 60, 70, 90 105 Gy using a Cobalt-60 gamma source at the Nuclear Institute for Food and Agriculture, Peshawar, Pakistan. Fricke dosimetry system was used to measure the dose received by the lot of insects. Post-irradiated pupae were allowed to emerge in standard 30 x 30 x 30 cm Bugdorm cages and continuously supplied with 10% wt:vol sucrose solution. Males were offered virgin females in a 1:1 ratio from the Swat strain after six days and allowed to mate for two days and then blood-fed with mice and allowed to oviposit in individual tubes. Egg hatch rates, larval mortality, adult emergence and longevity were recorded for all treatments including the control.

Results: Induced sterility of 100% was achieved with a target dose of 70 Gy. No significant reduction in longevity was observed with any doses, however, male emergence was significantly reduced in groups that received > 30 Gy dose of gamma radiation. Females irradiated at 90 Gy were not only 100% sterile, but also laid significantly less eggs than in the control and other doses below 90 Gy. Similarly, females irradiated with 105 Gy were not only 100% sterile, but all of them were infecund and laid 0 eggs.

Conclusion: It was found that the Swat strain of *Ae. aegypti* can be completely sterilized with 70 Gy gamma irradiation for successful application of sterile insect techniques.

Key Words: SIT, irradiation, *Aedes aegypti*, sterility, dengue in Pakistan, mosquito control

Abstract ID 28

An Eco-Bio-Social Approach to Apply to Control Dengue in Vang Vieng District, Vientiane Province, Lao PDR, a Southeast Asian Global Outreach Tourist Hotspot (2012-2014)

Somphone SOULAPHY

Ministry of Health, Vientiane, Laos, Email: *sphone59@yahoo.com*

Dengue is a vector-borne disease transmitted to humans by *Aedes aegypti*. Lao PDR situated in South East Asia is one among the affected by dengue. Nowadays, dengue prevention and control in the Lao PDR is emphasizing reduction of the mosquito vector populations.

Vientiane province is located in the middle of Lao PDR. The epidemic of dengue has occurred in this province every year. The number of patients of Vang Vieng is increasing. Dengue has become an increasingly important public health problem during the last years in VangVieng. The *National Dengue Prevention and Control Strategy* has reduced mortality (Case Fatality Rate of Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS)<1% countrywide by 2012).

Lao PDR purpose field site Vang Vieng is one of the worldwide tourist destinations with more than 148,696 tourist visits per year and where national and man-made systems overlap; therefore efforts to reduce risk of potential vector-borne diseases is very important. This contains the hypothetical model of the determinants of dengue transmission in Vang Vieng, which is based on ecosystem to human health.

The objective here was to identify the change in eco-bio-social determinants of dengue control. To develop inter-sectoral community-based approaches, sustainable changes in behaviour are required.

Methodology: A retrospective study was carried out during Jun, 2012 to June, 2013 in Vang Vieng District, Vientiane province based on a Knowledge, Attitude and Practices (KAP) survey. The KAP survey was conducted in May 2012 covering 640 respondents, which were randomly selected from 64 villages.

Results: Knowledge, Attitude and Practices (KAP) survey: The survey covered 640 respondents selected randomly from 64 villages. Of these 57.3% were females and 42.7% males. The mean age was 40.7 years (SD=12.9). The main findings are as follows:

1. Knowledge that dengue is a disease of concern that cannot be prevented at 21.7%.
2. Vector related knowledge: Only 46.9% of respondents knew that mosquitoes transmitting DHF feed on daytime.
3. Perception of DHF susceptibility: Only 2.2% had understanding that children get DHF in the raining season.

4. Perception of DHF severity: Only 46.6% answered that high fever and severe headache are main symptoms of DHF.
5. Perception of benefit to prevent DHF: 41.7% knew that destroying mosquito breeding places is a difficult method to prevent the disease.

Conclusion: The research evaluated the outcome of the dengue prevention and control project in Vang Vieng. It involved evaluation of the prevention behaviour by households, which is associated with knowledge of dengue, and perception and participation in activities to prevent dengue. The number of dengue cases was reduced after the intervention.

Abstract ID 43

Integration of Conventional Vector Control with Sterile Insect Technique against the Dengue Vectors *Aedes aegypti* and *Aedes albopictus* in Pakistan

Gul Zamin KHAN[1], Inamullah KHAN[1], Alam ZEB[1] and Tahir BADSHAH[2]

[1]Nuclear Institute for Food and Agriculture (NIFA), Pakistan Atomic Energy Commission, Peshawar, Pakistan, Email: *alamzebnifa@gmail.com*
[2]University of Haripur, Khyber Pakhtunkhwa, Pakistan

Background: Dengue is a deadly disease with alarming spreading potential that needs proper attention. As no vaccine for the disease is available globally, vector control is the only option in the present scenario. The use of insecticides for vector control have environmental constraints, health hazards and resistance development in mosquitoes, thus integration of conventional control methods with environmentally-friendly vector control strategies, including the Sterile Insect Technique (SIT), are needed.

Methodology: Sex separation of target species plays an integral part in any mosquito SIT programme. At NIFA, Peshawar, we tested the idea of protein and carbohydrate enriched diets and their effect on male / female differential development and sexual dimorphism in *Aedes albopictus* and *Aedes aegypti* under the ongoing IAEA coordinated research project.

In our efforts to integrate SIT with conventional methods of control, also some locally available eco-friendly plant extracts from *Piper nigrum*, *Curcuma longa* and bitter gourd (*Momordica charantia*) were evaluated against the 3rd and 4th instars larvae of *Aedes* mosquitoes.

Results: Results revealed that stevia 75% + bovine liver 20% + yeast 5% diet in mixture when fed to larvae produced 47% larger sized female pupae than in the control. However, the effect of these diet combinations did not favour the sexual dimorphism in other species. From the results regarding mechanical sex separations, mesh size of 1.25 mm separated both the sexes effectively with a mean accuracy range (97-100%).

Results also revealed that plant extracts (100 ml ethanol), (50 ml ethanol+ 50 ml tap water), (25 ml ethanol +75 ml tap water) and (100 ml tap water) when use in 2:2:1 ratio gave 100% mortality of tested mosquitoes. Thus environment-friendly plant-based pesticides could be integrated with SIT for population suppression.

Key words: Aedes aegypti, mosquito control, SIT, conventional control, dengue

Abstract ID 59

Ups and Downs during the Development of a Malaria SIT Programme for South Africa

Lizette KOEKEMOER[1], Givemore MUNHENGA[2], Oliver WOOD[2], Leanne LOBB[2], Leonard DANDALO[1], Basil BROOKE[2] and Maria KAISER[1]

[1]Wits Research Institute for Malaria, University of the Witwatersrand and the National Institute for Communicable Diseases, Johannesburg, South Africa Email: *lizettek@nicd.ac.za*
[2]National Institute for Communicable Diseases (NICD), Johannesburg, South Africa

Anopheles arabiensis is a major vector of malaria in South Africa and is responsible for seasonal malaria transmission. However, it is difficult to control this species using residual insecticides owing to its variation of feeding and resting behaviour. In addition to this, insecticide resistance has been reported in this species and although the impact is not yet operationally visible.

Although South Africa aims for elimination, additional vector control strategies are urgently needed to strengthen current control interventions and the use of the sterile insect technique (SIT) amongst others is presently being investigated.

A summary of the research and activities during this investigation will be presented.

Abstract ID 63

Releases of Fungus Exposed *Aedes aegypti* Males Integrated with Sterile Insect Technique for an Area-wide Vector Management Programme

Mario A. RODRIGUEZ-PEREZ[1,2], Alfonso J. GARZA-HERNANDEZ[1] and Filiberto REYES-VILLANUEVA[1]

[1]Instituto Politécnico Nacional, Reynosa, Tamaulipas, Mexico, Email: *drmarodriguez@hotmail.com*
[2]Centro de Biotecnología Genómica, Reynosa, Tamaulipas, Mexico

Background: Earlier, we demonstrated the auto-dissemination of *Metarhizium anisopliae* through transfer of conidia from *Aedes aegypti* males to females during mating under semi-field conditions (doi:10.1371/journal.pntd.0004144).

Here, we present the field trials using releases of fungus-exposed males (RFEMs) and discuss how this can be integrated with sterile vectors for an area-wide integrated vector management (AW-IVM) programme.

Methodology: We collected key information from the mosquito-copula-fungus model, to use it as a platform and to examine the effect of RFEMs on wild mosquitoes. Two experimental groups were established: 15 households with RFEMs (treated area), and 15 households with the release of healthy males (control area). Before each release, the households in both areas were inspected and cleaned of larval breeding sites, in addition to being treated with intra-domicile chemical insecticides three weeks before releasing.

We made weekly releases in April-May (spring) and October-November (autumn) in 2015 and 2016 at the same plot (Nuevo Amanecer neighbourhood) in Reynosa, Mexico and rotated areas as treatments between releases to reduce unknown sources of error associated with households.

Results: In 2015, we released males in a ratio of 1: 1 and 5: 1 (males: females captured in human landing collections / household / week). There was no effect. Therefore, in 2016, we released 40 males / household: 20 indoors and 20 outside, in front of the door. In the spring, natural populations were observed during the first five weeks, and then began the RFEMs from week 6th through 12th. A control area using release of healthy males was also included. At week 12th, populations began to decline because of low temperatures; from week 7th through 10th, the density of females in treated areas was significantly lower than that of the control area.

In autumn, 40 males / household were released from the beginning (week 1) in the treated area and the release of healthy males in the control area. We recorded that from the 3rd week, the densities of females captured using human landing collections were different. Females' average per household in the control area, at week 8, was 4.53, while it was 2.33 per household in the treated area. Variation was highly significant.

Based on entomological components of the Ross-McDonald model that we examined earlier (doi: 10.1371/journal.pntd.0002013), the vector capacity (C) analysis showed that in the treated area, the C decreased more than 278 times compared to that of the control area. The C in control and treated area was 1.11 infectious bites / day and 0.004 infectious bites / day, respectively.

Conclusions: RFEMs and sterile *Ae. aegypti* male technique are compatible as both require a facility for mass-rearing. The RFEMs technique also needs fungi which are cheaply produced by using natural substrates in plastic bags, to have a low cost production in an IVM control programme for dengue in any developing country.

An IVM control programme of this kind can prevent new infectious bites as well as reduce the number of vectors over a wide area endemic for dengue and other arbovirosis.

Abstract ID 70

Development of Sex Separation Tools to Eliminate Female *Anopheles arabiensis* during Mass-production

Givemore MUNHENGA, Lizette KOEKEMOER, Basil BROOKE and Thabo MASHATOLA

Wits Research Institute for Malaria and National Institute for Communicable Diseases, Vector Control Reference Laboratory, Johannesburg, South Africa, Email: *givemorem@nicd.ac.za*

Over the past six years South Africa has optimised various technical aspects of the sterile insect technique (SIT) for control of the malaria vector *Anopheles arabiensis*. One of the critical steps needed for applying a mosquito SIT is having a sexing system to eliminate female mosquitoes from the production line before irradiation and field releases. Female mosquitoes cannot be released, because of their capacity as disease vectors. Additionally male only releases increase SIT programme efficiency because released sterile males will only focus on mating with wild females.

Our group is exploring the use of classical genetics to accomplish female elimination in a laboratory *An. arabiensis* strain targeted for use during a planned SIT pilot release in northern KwaZulu-Natal Province, South Africa.

During this conference information on the successful development of an *An. arabiensis* genetic sexing strain utilising dieldrin resistance as a selectable marker, while containing a South African genetic background, will be presented.

Results on both the reproductive and physiological fitness as well as strain stability will be presented and discussed. Data on the effectiveness/efficacy of other environmentally-acceptable insecticides targeting GABA receptors in eliminating females from the newly developed strain will also be presented.

In view of the challenges being faced in using the new sexing strain results on alternative sex-sorting systems based on (i) blood feeding behaviour of adult females, and (ii) sex linkage using a selectable temperature sensitivity gene in which temperature conditions favour the production of males only will also be presented.

Abstract ID 73

Pilot Test to Assess the Effectiveness of SIT + IIT to Suppress *Aedes aegypti* Populations in Chiapas, Mexico

Pablo LIEDO-FERNANDEZ[1], Ildefonso FERNANDEZ-SALAS[2], Guillermo BOND[2], Carlos F. MARINA-FERNANDEZ[2] and Arianne DOR[1]

[1]El Colegio de Frontera Sur (ECOSUR), Tapachula, Mexico, Email: *ildefonso.fernandez@insp.mx*
[2]Centro Regional de Investigación en Salud Pública (CRISP), Instituto Nacional de Salud Pública, Tapachula, Mexico

Our aim in this project is to assess the effectiveness of the Sterile Insect Technique (SIT) plus the Incompatible Insect Technique (IIT) for the suppression of *Aedes aegypti* populations in pilot sites in Chiapas, Mexico.

Two sites of about 30 hectares each with similar environmental conditions have been selected near Tapachula city. Populations of *Aedes* mosquitoes have been monitored since October 2015 using ovitraps inside and outside of 15 houses for each locality. BG sentinel traps and GAP traps will also be used for monitoring of adult populations.

Mark-release-recapture method will be used to evaluate population size and dispersal of *Ae. aegypti* adults. Five transects around each community have been set in the surrounding vegetation zones. Traps are located at 0, 50 and 100 m from the village perimeter. Their objective is to evaluate the spatial distribution of *Ae. albopictus* and *Ae. aegypti*.

After 18-24 months monitoring, one site will be selected for sterile male mosquito releases, and the other site will be used as control. After one year, the sites will be switched, with the purpose of have one year of releases in each site.

A genetically diverse colony was established at the laboratory, collecting wild mosquitos from 12 sites along the coast of Chiapas. Egg samples of this colony were sent to the FAO/IAEA Laboratory at Seiberdorf, Austria to produce a *Wolbachia* infected strain. Our goal is to characterize this new strain, to evaluate its performance under field cage conditions and to mass-rear it for field releases. Larval diets were also tested along with irradiation doses on pupal populations. Preliminary results are presented.

Abstract ID 92

SIT Project in Mauritius: Achievements, Lessons Learnt and the Way Forward

Ambicadutt BHEECARRY, Diana IYALOO and Khouaildi Bin ELAHEE

The Vector Biology and Control Division, Ministry of Health and Quality of Life, Curepipe, Mauritius, Email: *abheecarry.vbcd@govmu.org*

Background: Since 2012, Mauritius embarked upon a national tehnical cooperation project (MAR5019) aimed at investigating the possibility of controlling the population of *Aedes albopictus* in a pilot site (Panchvati) in Mauritius, integrating the Sterile Insect Technique.

Methodology: Ovitrap surveys were conducted on a weekly basis in Panchvati and Pointe des Lascars (a control site). Ovitrap productivity and viability of field-collected eggs were determined. Furthermore, competitiveness studies were also carried out in semi-field conditions, while two mark-release-recapture (MRR) trials were conducted in the village to investigate fitness of irradiated mass-produced males under field conditions.

Results: Results from the weekly ovitrap surveys (Figure 1) as well as the MRR trials (Table 1), indicate an important wild population of *Aedes albopictus* living in Panchvati and in the surrounding region. Moreover, released males dispersed poorly within the village with 64 to 82 % of the total males recaptured being collected within a radius of 40 m from the release site.

As such, despite a ten-fold increase in the number of released males relative to the initial released number (5,000 sterile males/week) in Panchvati, a significant decrease in the vector incidence could not be achieved.

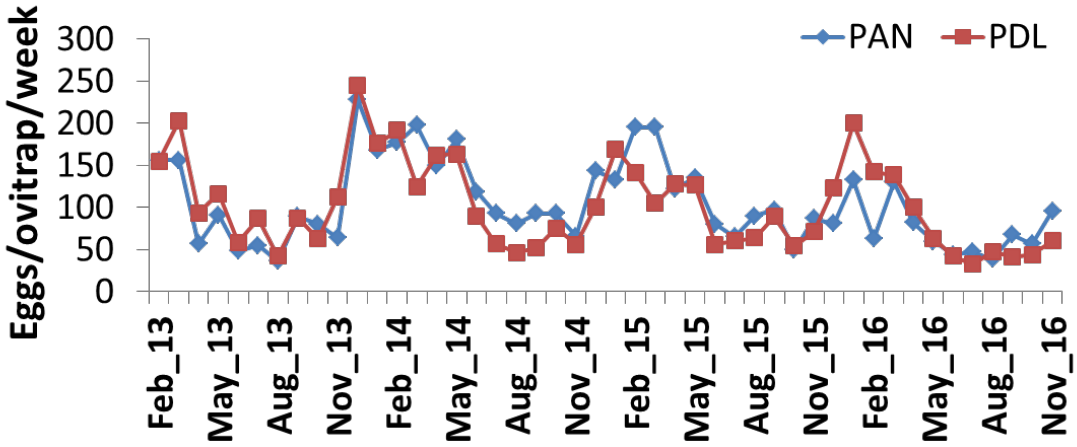


Fig. 1*: Ovitrap productivity.*

Table 1. *Results of mark-release-recapture trials MRR1 and MRR2.*

	MRR1	MRR2
Time of survey	July 2015	May 2016
No. of males released	3848	2200
Total recapture (%)	0.8	1.3
% recaptured within 40 m radius	64.3	82.1
Average Life Expectancy (days)	9	9
Estimated males per ha. (mean, 95 % CI)	2246 (1260 - 3232)	4095 (3358 - 4831)

Time of survey July 2015 May 2016 No. of males released 3848 2200 Total recapture (%) 0.8 1.3 % recaptured within 40 m radius 64.3 82.1 Average Life Expectancy (days) 9 9 Estimated males per ha. (Mean, 95 % CI) 2246 (1260 - 3232) 4095 (3358 - 4831)

Conclusion: Observations made during this study support the idea that SIT cannot be used as a stand-alone method, but can nevertheless be the principal component of an Area-wide-Integrated Vector Management (AW-IVM) strategy. Consequently, Mauritius still yearns for a successful SIT programme through harnessing lessons learnt and improving its next national project (cycle 2018-2019) by strengthening Integrated Vector Management components such as pre-sensitisation of the community towards proper management of waste/larval breeding sites, environmental management and chemical control, to quash the mosquito population to levels that are amenable to control by SIT.

Abstract ID 122

Solidary Nets that Prevent the Entry of Mosquitoes into the Houses

Gabriela WILLAT and Soledad ACUÑA

Ministry of Health, Montevideo, Uruguay, Email: *gwillat@msp.gub.uy*

Background: Vector-borne diseases account for 17% of the estimated global burden of infectious diseases. Among the diseases transmitted by mosquitoes are dengue, chikungunya, Zika fever, St. Louis encephalitis, malaria, yellow fever and West Nile virus fever. The vector-borne disease with the highest growth in the world is dengue, whose incidence has multiplied by 30 in the last 50 years.

The vector *Aedes aegypti* was eradicated from the country for 39 years. In 1997 its reintroduction was confirmed, and from that presence began work to generate a greater awareness in the population about the necessity of the elimination of mosquito larvae breeding sites.

After 20 years of cohabitation with *Ae. aegypti*, Uruguay faced the first dengue outbreak in 100 years in February 2016. *Ae. aegypti* is clearly an urban and domestic mosquito. It lives in our homes, and therefore, to avoid the diseases it transmits, we must protect ourselves from its bites.

Methodology: The aim is to assemble a digital communication campaign that invites people to donate their plastic containers to transform them into nets. People will post their donations and a government's brigade will pick them up and, after the recycling process, will deliver the mosquito nets to those in most need (hospitals, schools, etc.) Therefore, we propose the manufacture of mosquito nets from the recycling process of the different types of plastic that can be found at any home. Usually, house objects accumulating water are in disuse: buckets, tupperware, bottles, basins, etc. For this reason we suggest the donation of these containers to recycle them, turning them into plastic nets that will be installed to prevent entry into residences in need. We will work together with Sinergia Tech, technological development center and digital manufacturing laboratory in Uruguay, Montevideo's municipality, the Ministry of Social Development and the Ministry of Health to find innovative solutions.

Results: This action has multiple impact contributing to:
- Promote the theme "Uruguay against *Aedes aegypti*" in order to fit into a social agenda.
- Alter the population's behaviour, preventing habits acquired mainly at the time of emergency.
- Place nets as physical barriers between people and vectors, targeting homes of vulnerable populations.
- Protect ventilation apertures using mosquito nets, in order to reduce contact with the vector, acting as a physical barrier without damaging the environment.

Conclusion: The installation of these recycling plants (small and medium range) allows the transformation of potential breeding grounds into a product that protects the rooms, generating a solidary chain that emerges and promotes the work of the community.

This approach will fight the problem on several fronts, by eliminating obsolete containers that could be possible breeding grounds of the *Aedes aegypti* and by delivering mosquito nets to prevent the entry of mosquitoes into the houses.

Abstract ID 125

Implementation of a Surveillance Model for *Aedes aegypti* as a Support for Genetic Control Programmes

Luiza GARZIERA[1], Michelle PEDROSA[2], Fabrício SOUZA[1], Margareth CAPURRO[2] and Jair VIRGÍNIO[1]

[1]Biofábrica Moscamed Brazil, Juazeiro, Bahia, Brazil, Email: *jair@moscamed.org.br*
[2]Universidade de São Paulo, São Paulo, Brazil

Background: The triple epidemic (dengue, chikungunya and Zika) experienced in Brazil indicates that the current methods of *Ae. aegypti* control have not achieved the expected results, leading to an incessant search for new alternatives. At the "*International Meeting for the Implementation of New Alternatives for* Ae. aegypti *Control in Brazil*", which took place in early 2016 under the coordination of the Ministry of Health, the genetic control was considered as a "potential promising technology" to be applied as an additional tool to integrated vector control.

In this context, insect population monitoring is key to the success of an operational genetic control programme. This component makes it possible to detect the occurrence, spatial distribution and population dynamics of the vector in the area to be controlled. Thus, this work aims to present the implementation of an area-wide monitoring network for *Ae. aegypti*.

Methodology: The entire urban area of a Jacobina, a municipality of Bahia with 662 hectares and 21 neighbourhoods, was monitored with 'ovitrap' traps. A grid measuring 150 m x 150 m was set up on the map of the city in order to provide an ideal coverage by the traps. This resulted in a design consisting of a trap in the centre of each cell, and trap positioning adjustments were also made during installation.

In total, 400 ovitraps were installed, two at each sampling point (intra- and peri-domicile), at a density of 3.30 ovitraps per hectare. GIS (Geographic Information System) tool was used both in the implementation stage and in the development stage of the monitoring. Thus, all sample points were georeferenced, providing a better visualization of the spatial distribution of the points as well as the critical categorization of the vector infested areas.

The collections were carried out on a weekly basis by Moscamed technicians, with the use of motorcycles, which facilitated access to areas more quickly and economically. A backpack was adapted for the transport of materials and paddles. During 39 months of monitoring, eggs in paddles were counted and the Egg Density Index (IDO) and Ovitrap Positivity Index (IPO) were calculated.

Results: The results obtained from each sampling point were categorized according to the weekly average of eggs collected. Each category was represented by a colour, ranging from red (higher density) to green (lower density). Maps were generated, representing the

fluctuation of the egg density of the mosquito, resulting in an important tool for decision making, support which allows an easier interpretation of the population data of the vector.

The monitoring programme implemented followed all planned costs, logistics and efficiency expectations. However, the search for improvement and reduction of costs must be constant, and these are the main aspects on which Moscamed is currently focused.

Conclusion: This surveillance programme can be used as a basis for other suppression projects that use of sterile insect technique and related technologies, as well as to contribute to the health surveillance efforts of municipalities.

Abstract ID 135

Recent Achievements in the Application of SIT Methods for the Suppression of *Aedes albopictus*

Romeo BELLINI, Arianna PUGGIOLI and Marco CARRIERI

Centro Agricoltura Ambiente (CAA) "G.Nicoli", Crevalcore, Italy, Email: *rbellini@caa.it*

Background: Aedes urban mosquito species, mainly *Ae. albopictus* and *Ae. aegypti*, are the main vectors of important human diseases such as dengue, chikungunya and Zika, impacting the health of hundreds of millions of people in tropical and sub-tropical regions. Mosquito density reduction is considered a necessity to prevent or reduce the epidemiological risk. Unfortunately due to their specific biological features, urban mosquitoes result operationally extremely difficult to control at a sufficient level using conventional methods: larval reduction through larval control and source reduction, community participation and adult control. Moreover the use of chemical insecticides for larval and adult control is becoming problematic because of the possible impact on the environment and the rise of resistance in target populations. Thus there is the urgent need to develop more effective alternative vector control methods.

Methodology: A mass-rearing unit with a production capacity of 100,000 *Ae. albopictus* sterile males per week was established at the CAA headquarters. Basic technology requirements were developed in collaboration with the FAO/IAEA. The sexing method exploits the sex dimorphism of pupae with a male pupae productivity in the range 22-28% (calculated on the total number of reared males), with a residual presence of females in the range 0.3-1.0%. In case a further reduction in the presence of residual females is required, the productivity of the male will decrease considerably. Sterilization is performed on aged male pupae in water by applying a radiation dose of 35 Gy, which produces sub-sterile males with 1-2% residual fertility.

We developed simple semi-field enclosures useful to perform competitiveness studies to evaluate performances of sterile males vs. fertile males. Several pilot field releases were also conducted to evaluate possible release methods, the sterile male release dose and timing, and the impact of sterile males on the local population.

Results Trials conducted in semi-field large enclosures showed highly variable results between replications making the model time consuming and not very sensitive to strain comparisons. The best performing radio-sterilized males showed a very high competitiveness index (0.96±0.62). Pilot field trials showed that sterile males released at the dose of 900-1,500 males/ha/week during the season may induce a significant sterility level in the local population. In addition, when the sterility in wild collected eggs achieved values in the range of 70-80%, a similar reduction was found also in the number of eggs in the ovitraps. But the

decline registered in the fertility of the local population was not enough to bring the population density to collapse.

Conclusion: Better performing methods for sex separation and sterile males release should be developed to increase the cost-effectiveness of the SIT strategy. Concerning sex separation, *Ae. albopictus*, as well as other species, show natural protandry which is under investigation for the potential it might offer to increase sex separation efficiency. The sterile male release system is particularly important in urban settings to achieve a homogeneous distribution of sterile males.

Abstract ID 144

Insecticide Resistance in *Aedes aegypti* Populations in Mexico

Jesús Felipe GONZÁLEZ-ROLDÁN, Fabian CORREA-MORALES and Gustavo SANCHEZ-TEJEDA

Centro Nacional de Programas Preventivos y Control de Enfermedades (CENAPRECE), Ministry of Health, Mexico City, Mexico, Email: *jesus.gonzalez@salud.gob.mx*

Background: In Mexico, vector-borne diseases are of public health concern due to their impact on morbidity and mortality. The use of insecticides against adult mosquitoes is one of the most effective ways to control vector population density. However, the massive and extensive use of insecticides has resulted in the development of resistance to insecticides. This study shows the results of a nation-wide assessment of susceptibility to pyrethroids, carbamates and organophosphates in wild populations of *Aedes aegypti* mosquitoes.

Methodology: Seven pyrethroids, two carbamates and two organophosphates were evaluated. The Center for Disease Control and Prevention (CDC) insecticide susceptibility test was performed on field-collected mosquitoes from 62 localities covering 24 states (all endemic regions for dengue). Mosquitoes were exposed to insecticides for 2 hours and mortality was determined after a 24-hour recovery period.

Results: Carbamates were the most effective group of insecticides (using World Health Organization criteria), which gave higher mortality and faster knockdown activity. Out of the 24 states we collected from, only one state showed resistance against carbamates. Organophosphates showed knockdown activity and mortality in 22 states. For pyrethroids, a lower susceptibility and highest mosquito recovery after initial knockdown were found in all of the 24 analysed states.

Conclusion: Insecticide resistance in populations of *Ae. aegypti* in Mexico is a serious problem facing control operations. Resistance to pyrethroids is a serious problem in 24 states. However, carbamates and organophosphates can still be used for effective mosquito control. To help prevent or delay resistance in vector populations, a system of permanent surveillance and integrated control strategies is recommended.

Abstract ID 147

Effects of Prolonged Colonization on the Biology of *Anopheles gambiae*

Michael OSAE, Emmanuel BAAFI and Charles Emmanuel ANNOH

Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, Accra, Ghana, Email: *mosae5@yahoo.com*

Background: A critical essence of mass-rearing insects for the sterile insect technique (SIT) is to ensure that colonization does not result in genetic divergence between colonized and wild populations from which they originated. This can lead to loss in competitiveness and at times incompatibility between colonized males and natural females targeted.

This study investigated effect of prolonged colonization on physiological and reproductive parameters that are linked to competitiveness and compatibility in an *Anopheles gambiae* strain which has been under colonization for several years.

Methodology: Wild *An. gambiae* were collected as larvae from breeding sites around Redco flats at Madina, a suburb of Accra, between May and September 2013. Biological parameters including: developmental time, adult body size, genitalia rotation, swarming and mating were compared between the wild collected specimens and a laboratory strain colonized in 2008 from the same locality. All experiments were carried out under standard insectary conditions.

Results: The laboratory strain took significantly longer time (11 days) to develop than the wild one (8 days). Adult sizes of the laboratory strain were significantly larger than the wild collected counterparts. There was no significant difference in the rate of genitalia rotation as a proxy for sexual maturation.

Peak swarming times were longer (> 5 minutes) for the laboratory strain compared with < 3 minutes for wild strain. In all possible crosses between the two strains, males of the laboratory strain inseminated more females (>77%) compared to the wild males (<58%).

Conclusion: Prolonged laboratory colonization affected developmental time, male swarming and insemination success, but did not affect the rate of sexual maturation. Under laboratory conditions, the colonized males possess superior biological traits related to fitness and competitiveness. However, their mating performance in competition with wild males for wild females under field conditions needs further investigation.

Abstract ID 149

Preliminary Survey of Mosquito Species for Evidence of Local Feasibility of Sterile Insect Technique against Dengue Vectors in Kyaukse, Myanmar

Chi Htwe YIN ZAR[1], Ei Nandar CHAW[1], Thandar AYE[1], Nwe Aye TIN[1], Ko Lwin KO[1], Tun Yin WIN[1], Aye Khai AYE[1], Hanano YAMADA[2] and Chaw Su MYA NILAR[3]

[1]Entomology Laboratory, Biotechnology Research Department, Kyaukse, Myanmar, Email: *yinzarchi@gmail.com*

[2]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria

[3]Department of Medical Research, Anesakhan, Myanmar

Background: Recently, the global burden of mosquito-borne diseases has been increasing, however more species specific mosquito control is a complex and difficult undertaking. The sterile insect technique (SIT) could potentially be a useful additional tool in reducing the vector population. In Myanmar, there is a lack of an effective model for sustainable vector control, weaknesses in entomological surveillance and no vaccine available at present, and so vector control remains essential for the reduction of vector density below the disease transmission threshold.

Methodology: To evaluate the SIT feasibility in Myanmar, three potential field sites have been selected. The selected sampling sites were Thu Nge Taw Village (Site 1), Ka Tae Village (Site 2) and Myin Ka Bat Village (Site 3) in the Kyaukse Township of Mandalay Region in Myanmar. A community engagement meeting was held for the stakeholders in the villages and the types of mosquito breeding sites were also determined. Mosquito larva and pupa were collected from those sites in August and September, 2016 which are the rainy months in Myanmar.

Results: The common breeding sites found were particularly items such as rain-filled discarded food and beverage containers, domestic water storage containers, flower vases and any other water-filled abandoned containers. Mosquitoes found included *Aedes aegypti*, *Aedes albopictus* and other mosquito species. Among them, *Aedes aegypti* and *Aedes albopictus* were found at 26.17% and 10.7% in Site 1, 24.5% and 31.5% in Site 2 and 17.32% and 4.5% in Site 3. The sizes of the selected sites are 0.03 km^2, 0.110 km^2 and 0.043 km^2 respectively and they are isolated geographically from human dwellings not included in the target site. Moreover, there is no report concerning severe natural conditions such as typhoons, floods and earthquakes.

Conclusion: The preliminary survey on the selected sites indicated that local SIT application as part of integrated vector management for dengue vector control may be feasible in those sites in terms of presence of target species, manageable size, isolation and lack of adverse natural conditions.

Abstract ID 153

Entomo-virological Surveillance of *Aedes aegypti* in Mexico

Jesus Felipe GONZÁLEZ-ROLDÁN, Fabian CORREA-MORALES and Gustavo SANCHEZ-TEJEDA

Centro Nacional de Programas Preventivos y Control de Enfermedades (CENAPRECE), Ministry of Health, Mexico City, Mexico, Email: *jesus.gonzalez@salud.gob.mx*

Background: The entomo-virological surveillance in Mexico began in 2011, when the National Center for Diagnosis and Epidemiological Reference (InDRE) released to the whole country a nation-wide protocol for the identification and typing of dengue virus in biological samples captured in the field from mosquitoes *Aedes aegypti*.

In July of 2011, in the state of Guerrero, the collection of eggs (obtained by the monitoring with ovitraps) and of adult specimens of *Ae. aegypti* (collected indoors with the use of CDC backpack aspirators) was initiated, where it was possible to isolate mosquitoes infected with dengue virus.

In 2014, justified by the introduction of chikungunya virus to the country, a monitoring was carried out in Acapulco city, where the presence of mosquitoes infected with dengue virus and chikungunya virus were identified. Since 2015, this activity has been carried out in all the States of Mexico, to identify the circulation of dengue, chikungunya and Zika viruses in mosquitoes *Ae. aegypti*.

Methodology: The capture of mosquitoes is done with CDC backpack aspirators. Approximately 15% of the blocks where entomological surveillance is implemented with ovitraps are sampled. Once mosquitoes are captured they are placed in waxed vessels; pools will have approximately 25 live mosquitoes from block sampled and are tagged with collection data (locality, sector and block). Once in the laboratory, they are placed in the refrigerator at -4 ° C for 5 -10 min. in order to immobilize them (not to kill them) to carry out the taxonomic identification, separating by sex and species. The presence of dengue, chikungunya and Zika virus is identified by molecular assays (RT-PCR).

Results: This integrated vector monitoring system has allowed the identification of mosquitoes infected with dengue, chikungunya and very recently Zika viruses (Figure 1). This surveillance has been implemented throughout the country, taking it as an alternative to the sole focus of control actions to suppress population densities of the vector.

Conclusion: The mainly impact of this national surveillance system is that it allows focusing on the prevention and control of these diseases in the Mexican population, thereby lowering the costs that the suppression actions generate, and reducing the use of insecticides.

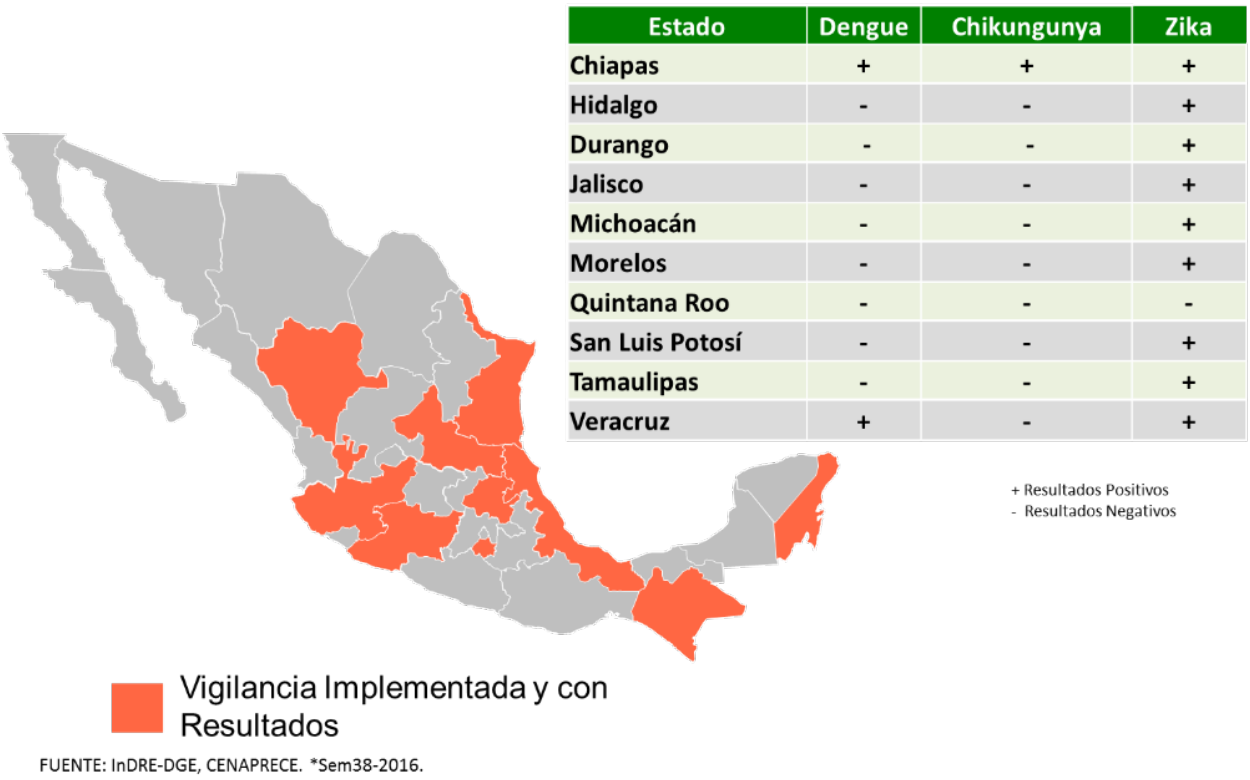


Fig. 1. *Resultados de la vigilancia entomovirológica en el país durante el 2016.*

Abstract ID 171

Screening for *Wolbachia* Infection in Mosquito and other Insect Populations in Ragama, Sri Lanka

Asha NAHALLAGE DONA[1], Y. I. N. Silva GUNAWARDENE[1], T. G. A. N. CHANDRASENA[2], R. S. DASSANAYAKE[3], P. A. D. H. N. GUNATHILAKA[2], Zhiyong XI[4], Kostas BOURTZIS[5], Wimaladharma ABEYEWICKREME[6] and Pattamaporn KITTAYAPONG[7]

[1]Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Sri Lanka
[2]Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka
[3]Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka
[4]Sun Yat-Sen University - Michigan State University Joint Center of Vector Control for Tropical Disease, Guangzhou, China
[5]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria
[6]Dengue Mega Project, National Research Council, Sri Lanka
[7]Center of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University at Salaya, Thailand

Background: In the absence of a 100% reliable sex separation method, the integration of the Sterile Insect Technique (SIT) with the Insect Incompatible Technique (IIT) is being considered as a promising strategy as it appears to be one of the most safe and sustainable method towards the population control of mosquito vector pests.

The SIT is based on the mass-rearing of a target species and its sterilization through ionizing irradiation, while the IIT is based on cytoplasmic incompatibility, which is a kind of male sterility induced by *Wolbachia*, a widespread maternally inherited bacterial symbiont of insects. Therefore, the information of *Wolbachia* strain variation and their properties within each insect species is important for the selection of *Wolbachia* strains to be used for applications of the combined SIT/IIT approach.

Methodology: As a preliminary survey, the prevalence of *Wolbachia* was investigated within natural mosquito and other insect populations in Sri Lanka. Insects collected from different parts of the country were morphologically identified at the species level and screened by conventional PCR assay. The DNA amplification was carried out by using primers which amplify the hypervariable regions (HVRs) of the *Wolbachia* surface protein (Wsp). PCR-positive insect specimen and species, producing an amplicon of 540~632 bp, were recorded. To address the evolutionary relationships between different *Wolbachia* strains, part of the wsp gene from positive samples was sequenced. Phylogenetic analysis [neighbour-joining (NJ)] was performed using the MEGA 4 software.

Results: One hundred and thirty different insect specimens belonging to 74 genera, 56 families and 5 different orders (Diptera, Lepidoptera, Odonata, Orthoptera and Hemiptera)

were screened for *Wolbachia* infection using the wsp gene PCR-based assay. Twenty-eight out of the 130 specimens were positive (21.5%). *Wolbachia* infections were more prevalent in Diptera, including 8 mosquito species, and less prevalent in Orthoptera. Percentage of divergence (DIST) between two species ranged from 0.0052 to 0.4933, while the wsp gene sequence analysis of 28 samples shared a 43%–99% homology with the wsp gene sequences in reference species.

Conclusion: *Wolbachia* infections are prevalent in mosquito and other insect populations in Ragama, Sri Lanka. Sequence and phylogenetic analysis based on the wsp gene suggest the presence of highly diverse *Wolbachia* strains, which could be exploited for applied purposes including the combined SIT/IIT approach for the population control of mosquito vector species such as *Aedes aegypti* and *Ae. albopictus*.

Abstract ID 177

Large Indoor Cage Study of the Suppression of Stable *Aedes aegypti* Populations by the Release of Thiotepa-sterilized Males

Rene GATO-ARMAS, Misladys RODRÍGUEZ, Rosa BRUZON, Ariamys COMPANIONI, Yanisley MARTÍNEZ, Zulema MENÉNDEZ, Natividad HERNÁNDEZ and Aileen GONZÁLEZ

Institute of Tropical Medicine Pedro Kouri, Havana, Cuba, Email: *renegato@infomed.sld.cu*

Background: Dengue prevention depends to a large extent on the control of the vector *Ae. aegypti*. The sterile insect technique (SIT) is a promising alternative to other available control methods. Here, we describe evidence of the feasibility of controlling *Ae. aegypti* by the release of chemo-sterilized mosquitoes.

Methodology: Stable colonies were established in each of the three cages during a 20 week period. Thiotepa-treated male pupae were released weekly into cages at a dynamic quantity needed to achieve 5:1 or 2:1 sterile : fertile male ratios. The number of pupae added to each treatment cage during the suppression phase was adjusted to reflect the impact of the thiotepa-treated males on the egg quantity and fertility. The hatch rate and adult female count were recorded weekly.

Results: The release of thiotepa-sterilised males at a 5:1 ratio with the fertile males was shown to be sufficient to eradicate a caged population of *Ae. aegypti* within 15 weeks. The introduction of thiotepa-treated males continued until the 5:1 release ratio treatment cage had produced no fertile eggs for three weeks, at which time the experiment was ended.

The hatch rate in the cage where sterile males were released at a 2:1 ratio exceeded 40% when the experiment was terminated, and the egg hatch and adult population (measured by adult catch during weekly trapping sessions) appeared to have stabilised by 10 weeks after the start of the suppression phase.

Conclusion: These results indicate that thiotepa exposure is an effective means of sterilizing *Ae. aegypti* and treated males were thus are able to reduce the reproductive capacity of a stable population.

Abstract ID 179

CRISPER Cas9-mediated Targeting of Dengue Viral RNA to Block Transmission in *Aedes aegypti* Mosquitoes - In-silico Approach

Hewawasam Patuwatha Badathuruge Kalindu Dulanja RAMYASOMA[1], Ranil Samantha DASSANAYAKE[2], Yasanthi Illika Nilmini Silva GUNAWARDENE[1], Wimaladharma ABEYEWICKREME[3], Menaka HAPUGODA[1] and Naduviladath Vishwanath CHANDRASEKERAN[2]

[1]Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Kelaniya, Sri Lanka, Email: *kd.ramyasoma@gmail.com*
[2]Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka
[3]Dengue Mega Project, National Research Council, Sri Lanka

Introduction: The genus Flavivirus of the family Flaviviridae includes several vector-borne viruses and dengue virus is one such virus having four serotypes (DENV-1,-2,-3 and 4). The clustered regularly interspaced short palindromic repeats/CRISPR-associated sequence 9 (CRISPR/Cas9) system is a self-dependent genome editing tool found in prokaryotes that acts as an adaptive immune system against foreign genetic elements.

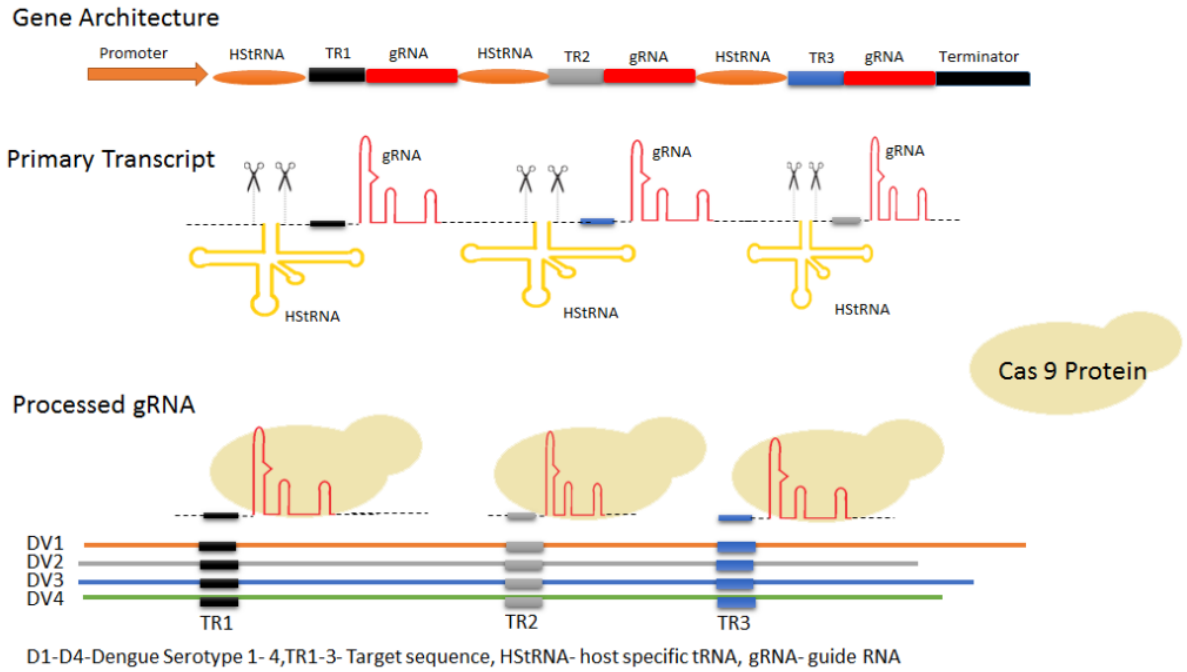
The Cas9 from the bacterium *Francisella novicida*, FnCas9 is a distinct RNA in eukaryotic cells and acts to eliminate the confounding interactions of FnCas9 with DNA and has been used to knockdown of the +ssRNA virus hepatitis C (HCV).

Methodology: Therefore, this mechanism can be adopted to knockdown dengue RNA to block the transmission having a proper genetic construct effective for four serotypes integrated into the mosquito genome.

In this study a multiple-CRISPR/ FnCas9 based genetic construct was designed in-silico to have multiple targeting sequences to be effective for all dengue serotypes.

Results: Multiple serotypes targeting sequences were engineered to co-express multiple gRNAs (guide RNA) carrying targeting sequences (TR) and interrupting tRNA separately. The exogenous cleavage of tRNA with RNase P and Z of this gRNAs will target dengue genome as shown the Figure 1.

Conclusion: The use of host-specific tRNA will ensure the separation of each gRNA and the architect gene ensures the multiple targeting of the dengue genome. However, to demonstrate the functional characteristics of in-silico designed CRISPR / FnCas9 gene construct capable of blocking dengue virus transmission, it is required to show function before germ line transformation into yellow fever mosquito *Ae. aegypti* or *Ae. albopictus* mosquito species. To ascertain this, CRSPR / CAS9 gene construct transformed into C6/36 (*Ae. albopictus*) or Aag2 (*Ae. aegypti*) cells infected with different dengue virus serotypes can be used.



D1-D4-Dengue Serotype 1- 4,TR1-3- Target sequence, HStRNA- host specific tRNA, gRNA- guide RNA

Abstract ID 181

A Combination of Mechanical and Behavioural Methods for Sex Separation of *Aedes* Mosquitoes for Sterile Insect Technique (SIT) and Incompatible Insect Techniques (IIT)

Wimaladharma ABEYEWICKREME[1], Tharaka RANATHUNGA[2], P. A. D. H. N. GUNATHILAKA[2], N. W. B. A. L UDAYANGA[2] and Asha NAHALLAGE DONA[2]

[1]Dengue Mega Project, National Research Council, Sri Lanka, Email: *wabeyewickreme@yahoo.com*
[2]Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Kelaniya, Sri Lanka

Background: Absence of an effective drug or vaccine for dengue has led health sectors to focus on the feasibility of integrating innovative vector controlling approaches such as the Sterile Insect Techniques (SIT) and the Incompatible Insect Techniques (IIT). Both these approaches require mass-rearing and releasing of vectors into the environment. As female mosquitoes are capable of transmitting the disease, total elimination of females from mass-releases remains to be critical in these approaches.

Methodology:
A. Mechanical separation using Fay and Morlan glass plates: Batches of 500 *Aedes aegypti* larvae were reared under normal and enhanced colony conditions. The emerged pupae from each colony were screened by using the Fay and Morlan glass plate separator. The separated sets of pupae from each colony were placed in separate cages and reared up to adults for morphological sex separation.

B. Behavioural separation using spiking blood meals with toxicants: Batches of 300 *Ae. albopictus* and *Ae. aegypti* mosquitoes with 1:1 male to female ratio was allowed to feed separately on spiked blood of cattle origin with different concentrations (2, 4, 6, 8 and 10 ppm) of Ivermectin (Ivotec,1% w/v) and Spinosad (Spinosyn,12 % w/v) separately. After 24 hours all remaining females and males were aspirated out and transferred into new cages separately. An additional blood meal with the initially fed concentrations of toxicant was provided after 24 hours, followed by a 48 hour observation of mortality. The experiment was repeated by feeding cattle blood containing a 1:1 mixture of each toxicant from each concentration.

Results: In the glass plate separation, 98.69% (n=227) of the males were separated in the first band together with 15.93% (n=43) of the females. The second band included the rest of the males (1.30%, n=3) and the females (84.07%, n=227). Under enhanced rearing conditions, 100% (n=240) of *Ae. aegypti* males were separated as the first band with 1.15% (n=3) of females. The second band included the rest 98.85% (n=257) of the females. Percentage of males and females separated at each band differed significantly ($p<0.05$) at 95% confidence level.

Spiking blood with 8 ppm toxicant killed all the fed females of *Ae aegypti* and *Ae. albopictus* within 24 to 48 hours. The number of fed females in both species gradually increased, with decreasing toxicity for blood with combined mixture. Male mortality did not show any significant difference during the study.

Conclusion: Fay & Morlan glass plate separator yielded a 100% separation of males with 1.15% female contamination under enhanced culture conditions, exhibiting its potential to be used as a sex separation method for *Ae. aegypti*. Double feeding of blood with 8 ppm Ivermectin and 8 ppm Spinosad showed the potential of using both toxicants as a viable treatment to eliminate female *Aedes* from laboratory colonies within 24-48hrs of feeding.

Use of Fay & Morlan glass plate separator, followed by spiking blood meals with Ivermectin/Spinosad to remove contaminated females, could be a potential method for 100% sex separation of *Aedes* mosquitoes for SIT and/or IIT.

Abstract ID 182

The Dose-rate Effects of Gamma Irradiation on Longevity, Sterility, Mating Competitiveness and Nix Gene of *Aedes aegypti*

Beni ERNAWAN[1], Usman Sumo Friend TAMBUNAN[2], Devita TETRIANA[3], Hadian Iman SASMITA[1] and Irawan SUGORO[1]

[1]Center for Isotopes and Radiation Application-National Nuclear Energy Agency, Jakarta, Indonesia, Email: *beni_e@batan.go.id*

[2]Department of Chemistry, Faculty Mathematics and Natural Science, University of Indonesia, Depok, Indonesia

[3]Center for Radiation Safety Technology and Metrology-National Nuclear Energy Agency, Jakarta, Indonesia

Background: Aedes aegypti is the most common mosquito vector for dengue, yellow fever, chikungunya and Zika viruses. Considering its medical importance, vector population control integrating the radiation-based sterile insect technique (SIT) is one of the potential methods for preventing and limiting the dispersal of these viruses.

The present study was undertaken to evaluate the dose-rates effects of gamma-irradiation on quality parameters of sterile males.

Methodology: Males *Ae. aegypti* at the pupal stadium were sterilized by applying 70 Gy of gamma-rays in various dose-rates, i.e. 0 (control), 300, 600, 900, 1200 and 1500 Gy/h utilizing panoramic Cobalt-60 irradiator. Adult males that emerged from the pupal stage were assessed for their quality parameters, such as longevity, sterility, mating competitiveness and DNA sequence analysis of Nix gene, the male-determination factor in the *Ae. aegypti* mosquito.

Results: The results indicate that the longevity of irradiated males was lower compared to control males. The data also demonstrated that longevity was significantly increased at the dose-rate from 300 to 900 Gy/h, then decreased at the dose-rate 900 to 1500 Gy/h. Sterility of irradiated males was significantly different compared to the control, while there was no significantly different at dose rate 300 to 1500 Gy/h. Mating competitiveness of irradiated males was increased at the dose rate from 300 to 1200 Gy/h, then the value decreased significantly at the dose rate 1500 Gy/h. The present study also revealed changes in the Nix gene, the *Ae. aegypti* male-determination factor, induced by the dose-rate of gamma-irradiation.

Conclusion: The dose-rate effects of gamma-irradiation are discussed in the context of genetic vector control, in particular the SIT. The results give information and contribute to a better understanding towards gamma-irradiation optimization and quality parameters of sterile male *Ae. aegypti* for SIT application.

Abstract ID 188

Aedes albopictus in Greece: Current State and Future Challenges

Antonios MICHAELAKIS[1], Nikolaos PAPADOPOULOS[2], Dimitrios PAPACHRISTOS[1], Panagiotis MILONAS[1] and Charalampos IOANNOU[2]

[1]Benaki Phytopathological Institute, Department of Entomology and Agricultural Zoology, Kifissia, Greece, Email: *a.michaelakis@bpi.gr*
[2]Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Magnisias, Greece

Since its first record, between 2003-2004 in western Greece (Ionian islands, Epirus), the invasive mosquito species, *Aedes albopictus* (Asian tiger mosquito) has been dispersed (up to 2016) all over Greece, except some areas of North Greece and islands in the Aegean Sea. Several of the new invaded areas that in the past experienced no intense mosquito annoyance, are now suffering because of the Asian tiger mosquito. Controlling moderate to high populations is challenging since intense chemical control efforts are of rather low efficacy.

In the current study, we report the invasion history of *Ae. albopictus* in Greece. The last three years, the EU funded project LIFE CONOPS has established a permanent network of trapping stations in Greece for the surveillance of *Ae. albopictus* and for the early detection of possible new arriving invasive mosquito species.

The goal of this strategy is to be able to proceed fast with immediate, emergency action to eliminate any newly established population before the invasive mosquitos disperse in large areas (as it was the case with *Ae. albopictus*). LIFE CONOPS has developed a list of operative management plans for invasive mosquito species that can be adopted by national, local and/or regional authorities that are responsible for mosquito management.

The southern parts of Greece are considered friendly for the establishment of the *Aedes aegypti* and other important tropical invasive mosquitoes. Therefore, it is of high importance to strengthen the surveillance of invasive mosquito species in areas at risk of entry or establishment and risk for disease transmission.

An area-wide community-based approach to control *Ae. albopictus* has been developed, addressing systematically all the breeding sites in private and public areas within a community.

Currently we are working towards developing a sterile insect release programme (SIT) to control *Ae. albopictus* populations in newly invaded areas that are still characterized by limited geographic dispersion and low population densities. The SIT programme will be incorporated and become an integral part of the area-wide strategy that we have developed.

Abstract ID 195

Establisment of the Asian tiger mosquito *Aedes albopictus* in Bulgaria: A New Challenge for the Mosquito Control Campaigns

Ognyan MIKOV[1], Ivelina KATERINOVA[2] and Tancho AGUSHEV[3]

[1]National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria, Email: *mikov@ncipd.org*
[2]National Diagnostic Science-and-Research Veterinary Medical Institute, Sofia, Bulgaria
[3]Agricultural University, Plovdiv, Bulgaria

The invasive Asian tiger mosquito *Aedes albopictus* was found for the first time in Bulgaria in 2011. The invaded area was the town of Sozopol in the Burgas region (south-eastern Bulgaria), located on the Black Sea coast and bordering Turkey. Despite the control efforts performed next year, the species was found breeding in the same location in 2012. Although the pathways and origin of introduction remain unknown, the closest countries having transport connections with Bulgaria and established populations of the species were Turkey, Russia, Georgia and Israel.

In 2013, mosquito trapping activities were conducted sporadically in Blagoevgrad and Plovdiv regions using BG-Sentinel traps with BG-Lure. For the first time *Ae. albopictus* was intercepted outside Burgas region in 2013 in the town of Petrich in Blagoevgrad region (southwestern Bulgaria) bordering Greece and Macedonia.

Since the start in 2014 of the "*National Programme for Prevention and Control of Vector-borne Infections in Humans in the Republic of Bulgaria 2014-2018*", a general mosquito surveillance network using light traps was established in 15 out of the 28 regions of the country. Catches were sent for identification to the National Centre of Infectious and Parasitic Diseases (NCIPD) on a monthly basis between April and October. Simultaneously, field collections were conducted by NCIPD in Blagoevgrad, Burgas, Vratsa, Montana, Pleven, Plovdiv, Haskovo and Silistra regions using BG-Sentinel traps and CDC light traps, as well as human landing collections.

In 2014, breeding populations were found in Blagoevgrad, Burgas, Vratsa, Plovdiv and Montana regions after receiving nuisance complaints from citizens. Larvae and adults of *Ae. albopictus* were confirmed in samples from the regions of Burgas, Montana and Stara Zagora. In the regions of Pleven, Haskovo and Silistra *Ae. albopictus* was not found.

In 2016, the regions of Blagoevgrad, Burgas, Montana, Plovdiv, Stara Zagora and Vratsa have established populations of the Asian tiger mosquito. The regions of Dobrich, Haskovo, Pleven, Razgrad, Ruse, Silistra and Varna remain free from invasive mosquito species.

In areas where it has spread, *Ae. albopictus* was found in settlements with an altitude up to 200 metres above sea level. Its establishment in different parts of Bulgaria shows both

distribution from one municipality to the neighbouring ones (in Blagoevgrad, Burgas and Plovdiv regions), and numerous independent introductions from different points of entry.

The attempts to control the populations of *Ae. albopictus* in all the affected regions were exclusively based on adulticiding and proved themselves ineffective so far. As all the adulticides registered for professional mosquito control are pyrethroids. It is a matter of crucial importance to find alternative and efficient ways to suppress or eliminate the populations of invasive mosquitoes. The integration of the sterile insect technique may offer a possible contribution to solve this problem.

Abstract ID 199

Mating Competitiveness of *Aedes albopictus* (Skuse) Males Exposed to Gamma-rays in Laboratory Cages; Towards Integrated Control through the Sterile Insect Technique (SIT)

Jeevanie Harishchandra RAMMANDA DEVAYALAGE[1], Wimaladarma ABEYWICKRAMA[2], Jeremie GILLES[3], Risintha PREMARATHNE[4] and Menaka Nalini HAPUGODA[2]

[1]Anti Malaria Campaign, Colombo, Sri Lanka, Email: *jeevanieharishchandra@yahoo.com*
[2]Faculty of Medicine, University of Kelaniya, Kelaniya, Indonesia
[3]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria
[4]Ministry of Health, Colombo, Sri Lanka

Background: To suppress population of *Aedes albopictus*, a vector of dengue in Sri Lanka, the Sterile Insect Technique (SIT) could be a potential method to be integrated with other conventional control methods. Assessing the mating competitiveness of irradiated sterile males and selecting optimum release ratios of sterile males were studied as these are key components of such programmes.

Methodology: Batches of male pupae (n=350) aged 28-40 hours in the F_8 generation of an *Ae. albopictus* laboratory colony were placed in plastic vials (height 7 cm, diameter 3.5 cm) with 10 ml of water and irradiated using a Gamma 220 (Cobalt-60) irradiator at the dose of 45 Gy. Dosimetry was performed using Fricke method.

Three experimental ratios were tested in laboratory cages (30 x 30 x 30 cm) after three days of emergence; sterile males: fertile males: virgin females 1:1:1, 3:1:1 and 5:1:1 (n = 100). Controls were set with fertile males or sterile males with 100 virgin females. Mosquitoes were allowed to mate with access to 10% sucrose solution. After three days, blood feeding was carried out 3 times for two days. All blood fed females were isolated into small tubes (height 7 cm, diameter 3.5 cm) with a wet filter paper lining the bottom for oviposition.

Egg papers were observed daily and filter papers with eggs were removed, number of eggs were recorded, and stored in the same tubes after draining water. Whenever, eggs are not laid after five days the females were removed into a cage and a second blood meal was given and replaced in tubes. Individual egg papers were hatched after one week in tubes using hatching solution (Nutrient Broth 0.25g, Brewers' yeast 0.05g, 750 ml distilled water). After 12 hrs number of L_1 larvae were recorded and hatch rates were calculated for individual females. The competitiveness of sterile males was compared by means of hatch rates of the three ratios tested (ANOVA, Post Hoc). Fried competitive index C was calculated using mean hatch rates.

Results: The mean hatch rates of fertile control, 1:1:1, 3:1:1, 5:1:1 and sterile control cages were 80.09±3.75SE, 50.81 ± 6.17SE, 31.78 ± 4.66SE, 21.18 ± 4.19SE, 6.25±1.3SE showing a decreasing trend as expected. Hatching rates of all tested ratios were significantly different from the fertile control (∞0.05%). The mean hatch rates were significantly different among the treatments except that the mean hatch rates between ratios 3:1:1, 5:1:1 was not significantly different, and more importantly the mean hatch Competitive Index C for the sterile male: fertile male: fertile female in 100:100:100 ratio was 0.66, indicating the irradiated males are 0.66 times as competitive as untreated males.

Conclusion: The results of the experiment shows positive trend towards the feasibility of using the Sterile Insect Technique as part of the integrated control of *Ae. albopictus*.

Abstract ID 211

Surveillance of *Anopheles arabiensis* Males Populations Based on Swarms Monitoring and Clay Pots Trapping Systems

Kounbobr Roch DABIRÉ[1], Simon P. SAWADOGO[1], B. Serge PODA[1], D. Dieudonné SOMA[1], N. Sévérin SOMDA[1], Hamidou MAIGA[1], Abdoulaye DIABATÉ[1], Gabriella GIBSON[2] and Jeremie GILLES[3]

[1]Institute de Recherche en Sciences de la Sante (IRSS), Bobo-Dioulasso, Burkina Faso, Email: *dabire_roch@hotmail.com*
[2]Natural Resources Institute, University of Greenwich, Chatham, UK
[3]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria

Background: The primarily goal of the SIT is to contribute to the suppression or elimination of existing vector populations in order to prevent or to reduce the disease burden. To achieve this objective, there are many aspects of the mosquito SIT package that still require increased efficiency to be deliverable at the operational level. Among them, male population surveillance systems are a crucial need, which allow monitoring the male populations (wild or released) in the field. This is possible if basic studies are performed on male ecology from swarming systems to resting habitats.

Many studies during the 10 past years have already gathered various knowledge on the swarming systems of *An. gambiae s.s.* (former M and S forms). But few data are available for *An. arabiensis* that is the targeted species by some SIT programme. The current study aimed to test different tools able to monitor *An. arabiensis* male populations during their resting period where they do not swarm.

Methodology: Males of *Anopheles arabiensis* were sampled within natural swarms around dusk following transect surveys during 10 consecutive days in December 2016 in Dioulassoba, a district of Bobo-Dioulasso city in Burkina Faso. The swarming points and places were then mapped throughout the transect. Twelve clay pots previously wetted in their inner linings were placed 2 to 5 meters apart from the swarming places indoor and outdoor of human courtyards. Each day they were visited to collect all mosquitoes resting, mostly in the morning from 6 to 9 am. In parallel indoor pyrethrum spraying catches (PSC) were also performed during ten days near the swarming sites. Ten outdoor resting sites such as hen roosts and wood piles were also sampled using electric aspirators. The data were then compared to evaluate the more efficient collection methods in comparison to the swarm sampling.

Results: The preliminary results showed that 116 mosquitoes sampled from 10 swarms were identified as 100% *An. arabiensis* males, whereas from the 12 clay pots sampled near the swarming points, 171 *An. arabiensis* mosquitoes were collected, of which 79% were males

and 21% females. Clay pots put within courtyards (outdoors) permitted to collect 79 mosquitoes (82% males vs 18% females), while only 21 mosquitoes were sampled indoors with only 43% males for 57% females. Furthermore the PSC method permitted to collect few mosquitoes (15), of which 40% were males and 60% females. The other outdoors resting sites yielded 57 mosquitoes (75% males).

Conclusion: The clay pot trapping method that is still under development showed very promising results that can complement the swarm sampling technique to track male populations in the field. However the results are very preliminary and we need to extend the study during at least 3 months to confirm the main tendency.

Abstract ID 227

Development of the SIT for *Aedes aegypti* in the Philippines: Colony Establishment, Larval Diet Evaluation and Population Monitoring

Glenda OBRA, Sotero RESILVA, Abigaile Mia JAVIER and Arvin DIMAANO

Philippine Nuclear Research Institute, Quezon City, Philippines, Email: *gbobra@pnri.dost.gov.ph*

An *Aedes aegypti* colony was established at the Philippine Nuclear Research Institute from collections made in Quezon City. Different larval diets were evaluated, based on various parameters, to determine the diet(s) that can be used for larval rearing and ensure feed availability for mass-production of the dengue mosquito vector, *Ae. aegypti*. Deviation from 1:1 sex ratio was also determined for each diet.

Based on pupal recovery, some diets produced more than 30% male pupae on Day 1 alone with only about 6-7% female pupae. The sex ratio was skewed in favour of males on these diets, which may be favourable in an SIT programme, where sterile males are used for release to mate with wild females.

Evaluation BG sentinel, vacuum aspirator and ovicidal/larvicidal (OL) trap in monitoring *Ae. aegypti* population was conducted in a selected pilot site in Quezon City. OL trap out-competed the two other devices based on trap collections in the pilot site from October 2015 to September 2016.

Abstract ID 237

Delivering Change: Potential Release Patterns and Distribution Systems for the Release of Transgenic Mosquitoes in Burkina Faso

Ferdinand HABBEL, John MUMFORD, Megan QUINLAN and Adrian LEACH

Imperial College London, London, UK, Email: *fhabbel@live.com*

Background: Malaria, transmitted by the *Anopheles* mosquitoes, killed more than 400,000 people globally in 2015. Estimates show that in Burkina Faso, a country of 17 million people, there were up to 10 million cases of malaria in 2015. Novel control methods, such as the release of transgenic mosquitoes, may provide additional options for vector control.

Efficient implementation planning requires information about mosquito release and dispersal, to design an appropriate distribution system, within developing countries, such as Burkina Faso. This MSc research project addressed these knowledge gaps by considering three questions:
Q1: How many transgenic mosquitoes need to be released at a release point for the release to be a 'success' and how many mosquitoes may need to be released in Burkina Faso?
Q2: What is the most effective pattern of transgenic mosquito release?
Q3: What are the implications of a centralised or decentralised distribution system in this context?

Methodology: A stochastic model was created to simulate three release patterns and subsequent mosquito dispersal in a short period after release. To input realistic data into the model, a method was developed to measure settlement characteristics, such as distance between settlements, the distance from roads and settlement size of settlements in Burkina Faso. In addition, a distribution model was developed to simulate the efficacy of centralised and decentralised distribution systems.

Results: The analysis indicates that a discrete rule-based release pattern is most effective in successfully establishing mosquito populations in settlements. Under such a release pattern, about six million mosquitoes need to be released to establish gene-drive populations in every settlement in Burkina Faso. Alternatively, a continuous release pattern may require the release of more mosquitoes, but would require less planning and logistics. Distribution of transgenic mosquitoes from a single distribution unit (centralised distribution system) would not be sufficient for a ground release strategy in Burkina Faso with ground releases, due to the constraints of mosquito chill duration and the length of a working day. This indicates that either aerial releases are required or ground based distribution from multiple, strategically-positioned, distribution unit (decentralised distribution system) would be necessary.

Conclusion: The findings of this research are particularly useful to identify necessary future field research, such as mark-release-recapture (MRR) experiments and mosquito biology

studies. Additionally, it will also contribute to specifications for the development of modes of transport, facilities and efficient distribution systems.

Abstract ID 239

Product or Service? Some of the Distinctions Arising from New Production Development in Mosquito Control

Megan QUINLAN

Imperial College London, London, UK, Email: *m.quinlan@imperial.ac.uk*

The push for new strategies in malaria vector control is supported by both the international position that what is presently available cannot maintain impact, and by the interest and support of private foundations. One initiative, a not-for-profit consortium aiming to reduce African malaria vector populations through genetic strategies, has been working through the distinctions of traditional area-wide field programmes versus development of a product that will be taken forward by others. While the Joint IAEA/FAO Division has a significant role in development of useful strains and methodologies, it also provides a range of services in cooperation with national representatives. Ultimately, however, the necessities of continual rearing and release of sterile insects has been managed more as a collaborative service, rather than provision of a specified threshold or outcome. An entity supported by project funding or shorter term resources must prepare products to stand alone more fully than might be needed in the intergovernmental treaty realm. Although the *Target Malaria* contributions are a long way from programmatic use, the author is completing a PhD on various tools and practices that will support employment of an "insect as a product" some years in the future. Some of additional requirements for a product approach are greater documentation, validation and traceability of colony origin and identity over time; best practices for partnerships and team building across different types of laboratories and research teams (discovery, proof of principle and delivery); greater harmonisation of and accessibility to data and cooperative ownership of results; and deeper and ongoing engagement with a range of stakeholders for whom area-wide control using a living product is a new paradigm. Malaria vector control also begs the question of how much a new product must prove efficacious against disease indicators, rather than mosquito population indicators. Participants in this process have acknowledged that shifting from a research objective to a product development one evoked a significant change in their working culture. It is important to not overrun the requirements for a modified insect as a product, however, for example by treating it as a pesticide, drug or vaccine. If relying on disease incidence as an indicator of efficacy, the burden of documentation and regulatory studies will hinder progress towards the health benefits desired by all.

In conclusion, both approaches of service provision and product development are legitimate for different scenarios. The dividing line is not so much traditional SIT vs novel genetic strategies, as it is the operational and political context, the technology available and the demands of funders. Societal values, such as concerns over genetic modification, may have

more impact on the best approach than the technology itself. No one approach to mosquito vector control will be sufficient.

Abstract ID 240

Assessment of Mosquito Presence and Density between Two Villages in Burkina Faso

Patric EPOPA[1], Azize MILLOGO[1], Matilda C. COLLINS[2], Ace NORTH[3], Samantha O'LOUGHLIN[2], Mark Q. BENEDICT[4], Abdoulaye DIABATÉ[1] and Roch K. DABIRÉ[1]

[1]Institut de Recherche en Sciences de la Santé (IRSS), Centre Muraz, Bobo-Dioulasso, Burkina Faso, Email: *dabire_roch@hotmail.com*
[2]Imperial College London, London, UK
[3]Oxford University, Oxford, UK
[4]Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA

Over the last decade there has been increasing interest in novel approaches to malaria vector control. Of the most promising are the genetic control approaches. To be effective, these will need to be sustainable and cost-effective, and for this there will need to be a good flow of constructs in space and time.

Until recently, *Target Malaria*'s field entomology activities have focused on the study of mosquito spatial and temporal dynamics inside villages. To predict the potential gene flow from one area to another, it is important to understand mosquito dynamics outside commonly sampled village areas and thus how constructs might flow between neighbouring villages.

This study aims to assess the presence and estimate the density of the mosquitoes in between two villages, Bana and Souroukoudingan, in Burkina Faso during the rainy season. The results show that mosquitoes do have a presence away from human habitation.

Future studies will extend the sampling further away from villages and will assess how much mosquito presence and density varies at different times of the year.

Abstract ID 241

The Use of Sequential Mark-Release-Recapture Experiments to Estimate Population Size, Survival and Dispersal of Male *Anopheles gambiae s.l.* Mosquitoes

Patric EPOPA[1], Azize A. MILLOGO[1], Matilda C. COLLINS[2], Ace NORTH[3], Frederic TRIPET[4], Mark Q. BENEDICT[5], Abdoulaye DIABATE[1] and Roch K. DABIRÉ[1]

[1]Institut de Recherche en Sciences de la Santé (IRSS), Centre Muraz, Bobo-Dioulasso, Burkina Faso, Email: *dabire_roch@hotmail.com*
[2]Imperial College London, London, UK
[3]University of Oxford, Oxford, UK
[4]Keele University, Newcastle, UK
[5]Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA

Background: Vector control is a major component of the malaria control strategy. The increasing spread of insecticide resistance has encouraged the development of new tools such as genetic control methods, which use the release of modified male mosquitoes. The efficient use of male mosquitoes as a control tool requires an improved understanding of male mosquito biology, including the factors influencing their survival and dispersal as well as the ability to accurately estimate a target mosquito population size.

Methodology: This study was designed to determine via repeated mark-release-recapture (MRR) experiments the seasonal variation in population size and to estimate the survival and dispersal of male mosquitoes of the *Anopheles gambiae* complex in a small West African village. Mark-release-recapture experiments were carried out in Bana Village over two consecutive years, during the wet and the dry seasons. For each experiment, around 5,000 (3407 - 5273) adult male *Anopheles coluzzii* mosquitoes were marked using three different colour dye powders (red, blue and green) and released in three different sites of the village (centre, edge and outside).

Results: Mosquitoes were recaptured at sites spread over the village for seven consecutive days following the releases. Three different capture methods were used: clay pots, pyrethroid spray catches and swarm sampling. Swarm sampling was the most productive method for recapturing male mosquitoes in the field. Population size and survival were estimated by Bayesian analyses of the Fisher-Ford model, revealing an about 10-fold increase in population size estimates between the end of the dry season (10,000 - 50,000) to the wet season (100,000 - 500,000).

There was no detectable seasonal effect on mosquito survival, suggesting that factors other than weather may play an important role. Mosquito dispersal ranged from 0-600 m above sea level and was not influenced by the season, but mainly by the release location, which explained more than 44% of the variance in net dispersal distance.

Conclusion: This study clearly shows that male-based MRR experiments can be used to estimate some parameters of wild male populations such as population size, survival, dispersal and to give an idea of the spatial dynamics in a given locality.

Abstract ID 264

Genetic Evidence for a World-wide Chaotic Dispersion Pattern of the Arbovirus Vector, *Aedes albopictus*

Mosè MANNI[1], Carmela R. GUGLIELMINO[1], Francesca SCOLARI[1], Anubis VEGA-RÚA[2], Anna-Bella FAILLOUX[3], Grazia SAVINI[1], Mariangela BONIZZONI[1], Ludvik M. GOMULSKI[1], Anna R. MALACRIDA[1] and Giuliano GASPERI[1]

[1]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy, Email: *giuliano.gasperi@unipv.it*
[2]Laboratory of Medical Entomology, Environment and Health Unit, Morne Jolivière, Institut Pasteur of Guadeloupe, Les Abymes, Guadeloupe, French West Indies
[3]Department of Virology, Arboviruses and Insect Vectors Unit, Institut Pasteur, Paris, France

Background: Invasive species represent a global concern for their rapid spread and the possibility of infectious disease transmission. This is the case of the global invader *Aedes albopictus*, the Asian tiger mosquito. This species is a vector of medically important arboviruses, notably chikungunya (CHIKV), dengue (DENV) and Zika (ZIKV). The reconstruction of the complex colonization pattern of this mosquito has great potential for mitigating its spread and, consequently, disease risks.

Methodology: Classical population genetics analyses and Approximate Bayesian Computation (ABC) approaches were combined to disentangle the demographic history of *A. albopictus* populations from representative countries in the Southeast Asian native range and in the recent and more recently colonized areas.

Results: In Southeast Asia, the low differentiation and the high co-ancestry values identified among China, Thailand and Japan indicate that, in the native range, these populations maintain high genetic connectivity, revealing their ancestral common origin. China appears to be the oldest population.

Outside Southeast Asia, the invasion process in La Réunion, America and the Mediterranean Basin is primarily supported by a chaotic propagule distribution, which cooperates in maintaining a relatively high genetic diversity within the adventive populations.

Conclusion: From our data, it appears that independent and also trans-continental introductions of *Ae. albopictus* may have facilitated the rapid establishment of adventive populations through admixture of unrelated genomes. As a consequence, a great amount of intra-population variability has been detected, and it is likely that this variability may extend to the genetic mechanisms controlling vector competence.

Thus, in the context of the invasion process of this mosquito, it is possible that both population ancestry and admixture contribute to create the conditions for the efficient transmission of arboviruses and for outbreak establishment.

Abstract ID 284

Procida Island (Italy, Campania Region): an Optimal Site for the Field Testing of Mosquito IPM Control Methods

Valeria PETRELLA[1], Luca IESU[1], Giuseppe SACCONE[1], Giuliano LANGELLA[2], Beniamino CAPUTO[3], Alessandra DELLA TORRE[3] and Marco SALVEMINI[1]

[1]Deptarment of Biology, University of Naples Federico II, Italy, Email: *marco.salvemini@unina.it*
[2]National Research Center, ISAFoM, Naples, Italy
[3]Department of Public Health and Infectious Diseases, University of Rome "La Sapienza", Italy

The Asian tiger mosquito *Aedes albopictus* is an invasive species well established in the Campania Region and its islands, in Southern Italy. Procida, the smallest island of the Gulf of Naples, has unique and very interesting features for field testing of mosquito integrated pest management methods: a very small size (3.7 Km^2), a completely urbanized and accessible territory, a high population density (more than 10,000 inhabitants) which doubles during summer because of tourism, and a strong presence of *A. albopictus*. Furthermore, Procida's worldwide famous image could help to easily get great media coverage in case of very positive population suppression results, and this could help in fund-raising efforts for future larger population control tests.

In April 2016, at the Department of Biology of the University of Naples Federico II, we started a one-year long pilot study of the local *A. albopictus* population in Procida to collect baseline data. With the help of the island administration we have actively involved the citizens of Procida as volunteers to monitor the seasonal and spatial distribution of this mosquito species.

Using 26 ovitraps, we collected a total of about 45K eggs over 12 months and we identified two egg density peaks in July and September 2016. During these two peak months, we analysed the spatial mosquito distribution using 101 ovitraps distributed all over the island territory, collecting about 40K eggs, with an average of eggs per trap of 100 and an ovitrap index > 90%.

We are also using environmental data collection and GIS to create a map of mosquito distribution in Procida. The collected data will be utilized to set up an *A. albopictus* sterile males release test on the island to be performed, in collaboration with the Joint FAO/IAEA Insect Pest Control Subprogramme, in the spring of 2018.

Our long-term and most ambitious objective is to try to obtain a consistent suppression of this invasive species from the island to help the local economy, which is mainly based on tourism, and to induce then the application of mosquito SIT-based control methods on other islands of Campania Region (Ischia and Capri), as well as on the mainland.

Poster Presentations Session 3: Animal Health

Abstract ID 7

Development and Application of Sterile Insect Technique (SIT) to Control/Suppress Blow Fly, *Lucilia cuprina* (Diptera: Calliphoridae) from Coastal Area of Bangladesh

Muhsina YASMIN, A. S. M. SAIFULLAH and A. T. M. F. ISLAM

Institute of Food and Radiation Biology (IFRB), Dhaka, Bangladesh, Email: *yasmin.muhsina@yahoo.com*

The blow fly *Lucilia cuprina* (Diptera: Calliphoridae) is a serious pest of sun-dried fish in the coastal area of Bangladesh. Its infestation of fish creates both qualitative and quantitative losses. The economic loss is estimated at up to US 1 million USD per year. Unlike insecticides, the current control method, the SIT acts as an effective and environmentally-friendly alternative for adequate suppression of blow fly population.

One of the principal steps of the SIT is mass-rearing. About 10 million pupae per week are produced at the Institute of Food and Radiation Biology using a cost effective artificial diet. Reproductive sterilization dose of mass reared pupae was determined as 45 Gy. Competitiveness of sterile flies was also patterned on a regular basis.

A total of seven test releases of sterilized flies were performed in the southern coastal fish drying yards. The results appeared to be very promising and it is clarifying the continuation of the SIT to suppress/control blow fly infestation from the coastal area of Bangladesh.

Abstract ID 25

Integrated Control of *Glossina palpalis gambiensis* in the Framework of its Eradication in the Northern Basin of Niger River in Mali

Aligui DJITEYE[1], Boucader DIARRA[1] and Issa SIDIBE[2]

[1]Direction Nationale des Services Vétérinaires (DNSV)/PATTEC, Bamako, Mali, Email: *addjiteye01@yahoo.com*
[2]CIRDES, Bobo-Dioulasso, Burkina Faso

In the execution of the Multinational Project activities "*Eradication of Tsetse and Trypanosomiasis in Sub-Saharan Africa", G. p. gambiensis* populations were reduced by 97.03 % in the northern basin of Niger River (18,000 km^2), 18 months after the installation of 9259 mono conical "Vavoua" traps impregnated with deltaméthrine at the dose of 300 mg / m^2 of tissue.

After the holding of sensitization and information meetings in the regional capitals, and the organization of "communal" workshops grouped 536 beneficiaries (leaders, elected representatives, technicians), 970 village sergeants were trained in the field of the impregnation, installation, surveillance and the removal of the traps, at the level of 191 concerned villages.

The reduction rates of the tsetse flies apparent density varied according to the part of the river, indeed the following average percentages were observed: 95.63 % on the right site (4 100 km^2), 98.49 % on the southwest part (2 300 km^2), 99.04 % on the left site (3 100 km^2), and 100 % on the north part (8 500 km^2).

Before control activities, a global trypanosomiasis prevalence rate of 2.80 % was observed in cattle. The causal trypanosomes species are *Trypanosoma vivax* (92.94 %) and *T. congolense* (7.06 %). The infection rates average observed at the sentinel herds' level are: 7.0 % on the right site, 1.3 % on the southwest part, 3.0 % on the left site and 0.5 % on the northern part. On 571 samples of bovine blood examined 3 months after control activities, a single positive case of pathogenic trypanosome was revealed, at an infection rate of 0.17 %.

Following the drastic reduction of *Glossina palpalis gambiensis* population density, it is intended to release sterile males (imported from CIRDES, Bobo-Dioulasso, Burkina Faso). The male adults were irradiated at 110 Gy in 1973; it is necessary to confirm that this dose does sterilize efficiently the descendants. A check of the optimal irradiation dose revealed that: at day 40 post-emergence the females mated with the control males produced 419 pupae, against 5 for the 110 Gy lot, 7 for the 125 Gy lot, 0 for the 140 Gy one, and 1 pupa for the females mated with 150 Gy irradiated males.

The former dose 110 Gy, retained to irradiate *G. palpalis gambiensis* males, does not inducted now 100 percent of infertility to the inseminated females. We have to investigate the

doses of 125 Gy or 140 Gy, further to competitiveness tests and mass-marking with fluorescent powder studies.

The continuation and the intensification of the installation of the impregnated traps and their surveillance by the village sergeants during the dry seasons, and the releases of sterile males during the next two rainy seasons, would allow eradicating *G. palpalis gambiensis* residual populations in the northern basin of the Niger River in Mali.

Abstract ID 68

High Level of Multiple Paternities in Wild *Glossina fuscipes fuscipes* Females in Buvuma Islands, Uganda

Agapitus KATO[1], Loyce OKEDI[2], Charles MASEMBE[3], Serap AKSOY[4], Gissela Caccone ADALGISA[5], Francesca SCOLARI[6] and Anna MALACRIDA[6]

[1]Ministry of Agriculture, Animal Industry and Fisheries, Entebbe, Uganda, Email: *agapituskato@gmail.com*
[2]National Livestock Resources Research Institute, Tororo, Uganda
[3]Department of Biological Sciences, College of Natural Sciences, Makerere University, Kampala, Uganda
[4]Division of Epidemiology of Microbial Diseases, Yale School of Public Health, Yale University, New Haven, USA
[5]Department of Ecology and Evolutionary Biology, Yale University, New Haven, USA
[6]Department of Animal Biology, University of Pavia, Italy

Background: Glossina fuscipes fuscipes is the main vector of human and animal trypanosomiasis in Uganda. Attempts to suppress/eradicate this species using biological methods require knowledge of its reproductive biology. An understanding of the patterns of sperm use and paternity skew can be important as they influence the effective population size and may constitute a critical factor in determining the success of control methods such as the SIT or the IIT (incompatible insect technique), or the replacement of disease-susceptible natural populations with modified resistant phenotypes.

Polyandry has been reported in *G. fuscipes fuscipes* in Uganda. Interest in assessing the presence of multiple paternities in Ugandan *G. fuscipes fuscipes* populations is driven by the fact that eradication of this species is at the planning stage in some areas of this country.

Methodology: We used microsatellite markers to investigate whether multiple paternities happen in wild (field-collected) *G. fuscipes fuscipes* tsetse fly females of Buvuma islands in Uganda.

Results: Our data revealed a minimum of 27% multiple paternities in wild females. When the mother is known, the likelihood-based simulation estimated 98.0% correct paternity assignment (Δ= 5.04; 95% confidence).

Conclusions: Control programmes against *G. fuscipes fuscipes* based on genetic methods should release abundant modified males, even within residual surviving target populations in the dry season.

Key words: multiple paternities, sperm use, Glossina fuscipes fuscipes*, microsatellites, wild populations*

Abstract ID 95

Biological Control of Livestock Pest Biting Fly *Stomoxys calcitrans* at Agriculture Pineapple Residues Using the Parasitoid *Spalangia endius* Reared on Irradiated Mediterranean Fruit Fly: Assessment of Parasitism in Field and Laboratory in Costa Rica

Jose Arturo SOLORZANO-ARROYO[1], Hazel MENA[1], Rigoberto ROMERO[2], Jairo TREVIÑO[3], Jeremie GILLES[4], Christopher GEDEN[5], David TAYLOR[5] and Henrik SKOVGAARD[6]

[1]Ministry of Agriculture and Livestock of Costa Rica, Agriculture Research Institute INTA, San José, Costa Rica, Email: *asolorzano@inta.go.cr*
[2]National Plant Health Office (SFE), Ministry of Agriculture and Livestock, San José, Costa Rica
[3]Pineapple Development Corporation PINDECO-Del Monte, Costa Rica
[4]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria
[5]USDA, Agricultural Research Service (USDA-ARS), Gainesville, Florida, USA
[6]University Aarhus, Aarhus, Denmark

Background: Stable fly, *Stomoxys calcitrans*, (Diptera: Muscidae) is a pest of high economic importance impacting the agriculture in Costa Rica. It also develops on the pineapple plant residues in the field becoming a national social and economic problem for dairy and beef farmers, representing the most important pest for livestock in the country. Outbreaks of more than 1000 stable flies/animal have been found affecting national livestock.

In order to find environmental solutions to the resistance development in insecticides used (IGR group), as well to improve tools for an IPM management, a project with the use of parasitoids as biological control agents was developed with cooperation of the IAEA under project COS5030 "*Supporting Biological Control of Stable Fly (S. calcitrans) through the Use of Parasitoids Reproduced on Irradiated Fruit Flies (*Ceratitis capitata*)*". The project aimed at evaluating the capacity of biological control to suppress the stable fly population through the use of specific parasitoids reared on irradiated Mediterranean fruit fly as host. This was achieved in partnership with the National Institute of Agricultural Technology Research (INTA) of Costa Rica and the collaboration of USDA-ARS in terms of the donation of an effective parasitoid *Spalangia endius* for developing the colony in Costa Rica.

Through the project, capacity building activities were organized to develop the capabilities at INTA to rear the parasitoid. This was done through the implementation of protocols to rear the parasitoid, and assessing the dosage for the irradiation process of the host larvae and assessment of the parasitism of *S. calcitrans* pupae. Rearing of the parasitoid was possible using irradiated *C. capitata* pupae as host from the Fruit fly Laboratory (SFE National Plant

Health Office) facilities in San Jose. The parasitoid colony was established at INTA´s agriculture research station Los Diamantes in Guápiles, Limón.

High temperature and relative humidity up to 100% became a problem in the colony as a result of which moulds and mites development happened. At the same time a development of a cross contamination with another parasitoid wasp (*Pachycrepoides vindemniae*) occurred at the fruit fly laboratory, which replaced almost most of the living *S. endius* parasitoids at the colony. Nevertheless, the project helped to provide knowledge to overcome these issues to rear a colony of stable fly at INTA`s laboratory for the life cycle management in the tropics.

Results: Stable fly parasitism assessments were carried out at laboratory scale and later under field conditions at different ratios of parasitoid wasps to *S. calcitrans* pupae (1: 6; 1:8; 1:10; 1:15; 1:20) and different depths (5, 10 and 15 cm) of pineapple residues (Figure 1). Parasitism rates ranged between 70% and 40% ($p<0,001$) between lab and field evaluations, with almost no difference in depth of search.

Conclusion: These encouraging results indicate that *S. endius* is an excellent parasitoid to incorporate into the management of *S. calcitrans* outbreaks, also because the assessment of the insecticide GRI Novaluron 10 EC indicated that it does not affect parasitoid mortality.

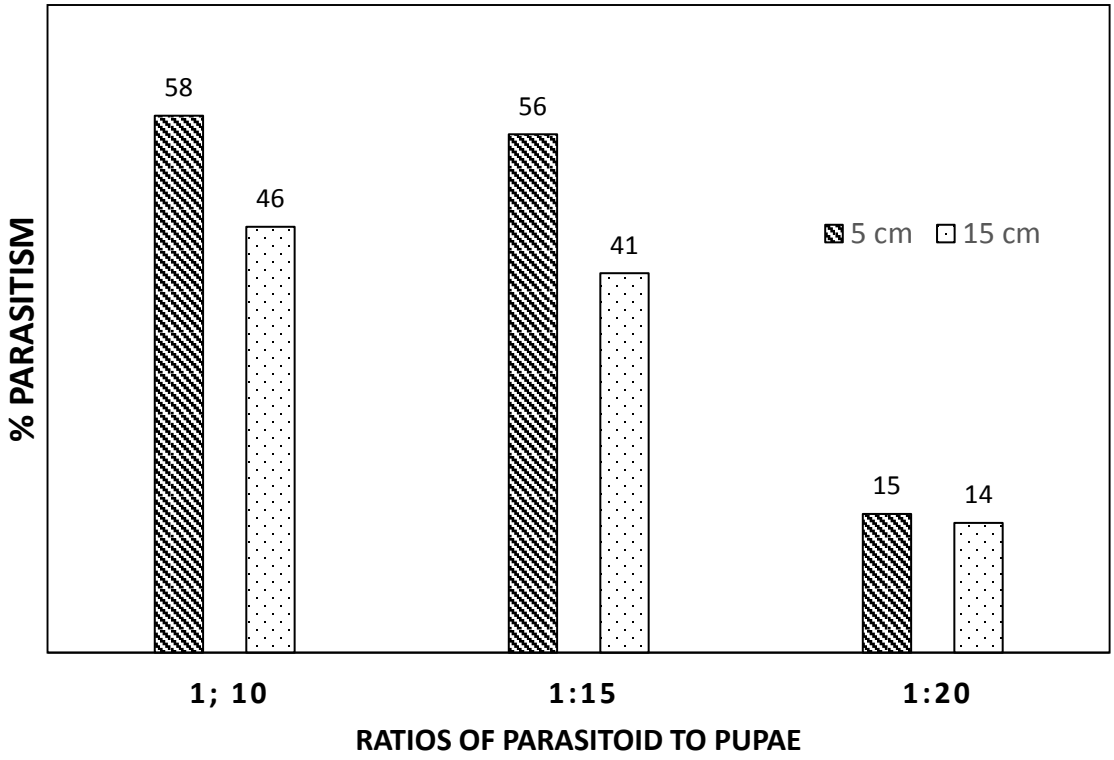


Fig. 1. *Percentage of field parasitism of* Spalangia endius *on* Stomoxys calcitrans *pupae at pineapple plant residues at two depths and three ratios of parasitoid wasps to* S. calcitrans *pupae.*

Abstract ID 103

2006-2016: 10 years of Tsetse and Trypanosomosis Control in Burkina Faso: What Achievements, Impacts and Perspectives in Terms of AW-IPM and SIT Application

Issa SIDIBE[1], Issa TAMBOURA[1], Mamadou OUEDRAOGO[2], Zowinde KOUDOUGOU[2], Lassane PERCOMA[1], Adama SOW[3], Hermann POODA[1], Soumaila PAGABELEGUEM[1] and Oumarou SERDEBEOGO[4]

[1]IBD-CETT, Bobo-Dioulasso, Burkina Faso, Email: *isidibe@hotmail.com*
[2]Ministère des Ressources Animales et Halieutiques
[3]EISMV, Dakar, Senegal
[4]Global Challenge, Bobo-Dioulasso, Burkina Faso

Since 2006, multidisciplinary activities have been conducted in order to create areas sustainably free from tsetse fly and trypanosomiasis in Burkina Faso. Different tools and strategies were developed:

(i) the use of GIS as a decision tool to select and map priority intervention zones
(ii) population genetics to identify isolated populations
(iii) entomology to assess the density of tsetse populations, and
(iv) characterization of trypanosome strains to assess trypanocidal drug resistance.

In terms of development, socio-economic impact of actions taken was evaluated halfway through this process. A socio-economic study conducted in 2012 showed, in regard of the pastoral economy: (i) reduced mortality by 60%, and (ii) a rate growth of 6% against a national average of 2%.

Strengthening capacities of technicians from public and private areas of the of livestock sector has improved very significantly the income generated. Increases generated in the pastoral economy are estimated between 14 and 120 million USD for just the cattle capital.

The current results of the project impact a population of about 1.5 million inhabitants, including farmers, ranchers, fishermen, women, children and livestock. More than 40,000 km^2 have been treated and sanitized. The combined actions of insecticide impregnated screens and spraying have reduced the tsetse pressure by nearly 95%.

The National Tsetse Control and Eradication Programme acquired more capabilities in addition to the facilities already in place (new Insectary built, ELAT rehabilitated and CIRDES) in order to boost the eradication process through an area-wide strategy that includes Sterile Insect Technique (SIT) implementation in Burkina Faso, and also support tsetse programmes in other infested countries in West Africa.

Abstract ID 114

Prevalence of Screwworm Myiasis due to *Cochliomyia hominivorax* in Newborn Calves in the Pantanal, Brazil

Antonio Thadeu BARROS, Ernande RAVAGLIA and Waldno AQUINO

Brazilian Agricultural Research Corporation (Embrapa), Ministry of Agriculture, Livestock, and Food Supply, Corumbá, Mato Grosso do Sul, Brazil, Email: *thadeu.barros@embrapa.br*

Background: The Brazilian Pantanal is a large seasonally flooded area bordering with Bolivia and Paraguay. With abundant wildlife, the main economic activity of this tropical wetland depends on extensive livestock farming, based on calf production in large ranches.

Navel myiasis caused by the larvae of the New World screwworm (NWS) *Cochliomyia hominivorax* (Coquerel) stands out among the most important diseases affecting new-born production in the region. Female screwworm flies are attracted to exposed and bleeding living tissue to lay eggs, and unhealed navels are excellent attractants to these flies. The ulcerative lesions caused by the NWS larvae favour bacterial infections and secondary myiasis by other fly species, and may lead animals to death if not treated. Indeed, umbilical myiases are considered a major cause of calf mortality in the Pantanal.

Methodology: The study was conducted at the experimental station of the Embrapa Pantanal, which produces from 300 to 400 calves in a birth season from September to December, during two consecutive birth seasons. New-born calves were monitored every 2 to 3 days until myiasis installation or complete navel healing was observed; parasitized animals were immediately treated when found. The study was carried out under routinely management conditions, allowing to determine the prevalence of navel myiasis in new-born calves in the region.

Observations were made in 236 calves during the whole study, being 141 in the first year and 95 in the second. Staff limitations and large breeding areas did not allow finding and treating the calves immediately after birth, so fewer new-borns with healthy navels were found in comparison to parasitized animals with screwworm larvae at different stages of development.

Results: The prevalence of umbilical myiasis was 91.2% in the first year and 92.5% in the following one. Such high prevalence of navel myiasis in new-born calves shows the severity of this parasitosis in the Pantanal. Such results confirm the importance of screwworm myiasis.

Conclusion: Despite the generally high efficacy of insecticide products and the continuous efforts of calf managing during birth season, many new-borns are lost due to NWS myiasis. The development and adoption of new approaches may help to prevent and control screwworm myiasis in the Pantanal, as well as in other critical areas of the country, thus reducing the economic losses caused by this parasite.

Abstract ID 117

Controlling the Spatio-temporal Dynamics of Tsetse Flies: Insights from a Mechanistic Model

Hélène CECILIA[1], Ahmadou DICKO[2], Sandie ARNOUX[1], Sébastien PICAULT[3], Jérémy BOUYER[4], Pauline EZANNO[1]

[1]National Institute of Agricultural Research (INRA), Nantes, France, Email: *helene.cecilia@oniris-nantes.fr*
[2]French Agricultural Research Centre for International Development (CIRAD), Montpellier, France
[3]Université de Lille, France
[4]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria

Background: Regional control programmes implemented in Africa in the last decades to eradicate tsetse flies (*Glossina* spp.), the vector of trypanosomes, resulted in contrasted outcomes. Some regions accomplished a successful eradication (e.g. in the Island of Unguja, Zanzibar), while others experienced a large decrease in tsetse population density followed by resurgence when the programme was stopped, indicating eradication had not been achieved (e.g. in Loos islands, Guinea). Therefore it becomes crucial to better understand how to target control efforts to sustainably eradicate tsetse populations from regions at high risk of trypanosomiasis. Our objective was to better understand the spatio-temporal population dynamics of tsetse flies and to identify the drivers of their control.

Mathematical models and computer-based simulations are relevant to assess which control measures should be used and when, accounting for the ecological complexity of the target pest population and territorial specificities of the controlled area. They provide a useful tool, complementary to field observations and experiments, to suggest efficient vector management strategies.

Methodology: We developed a deterministic and mechanistic spatio-temporal model of the population dynamics of tsetse flies, structured by sex and age (pupae, teneral and non-teneral adults). Temperature and fly density influenced the life-cycle, while spatial diffusion depended on density and relative quality of neighbouring locations.

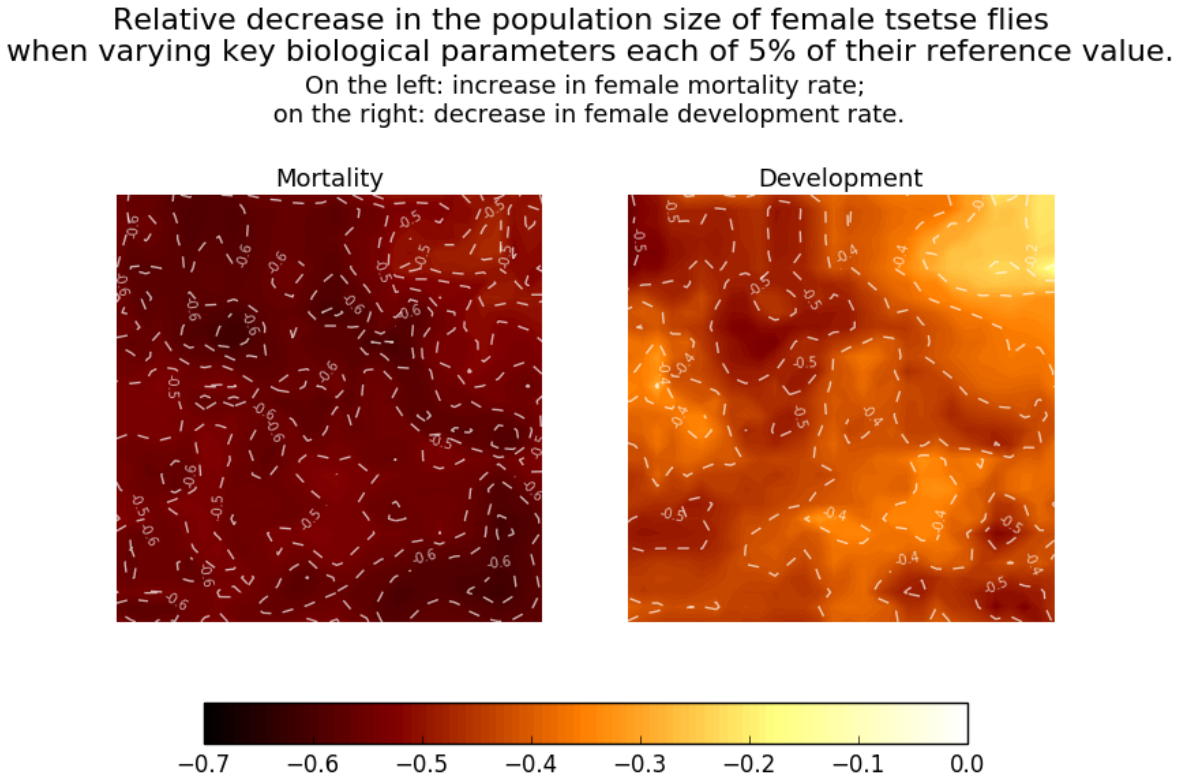
We applied the model on populations of *Glossina palpalis gambiensis* in the Niayes area of Senegal, for which biological and landscape data were available. The landscape was divided into 250 m x 250 m cells of heterogeneous carrying capacity estimated by habitat suitability models. We transformed observed temperatures into “perceived” ones, to account for micro-environments where flies live. Dispersal, mortality, and development rates were calibrated on laboratory data, experts’ opinions, and literature.

Results: The sensitivity analysis of the model identified the biological and environmental parameters influencing the most population dynamics. We showed that the mortality and development of adult females, along with temperature, were the key drivers of population persistence.

Our predictions suggest that combining techniques that both increase mortality and decrease fecundity would be optimal to eradicate tsetse flies in targeted zones (Figure). Sequential aerosol technique (SAT), traps and targets (TT) and insecticide-treated livestock (ITL) increase daily mortality rates, whereas the sterile insect technique (SIT), by preventing egg-laying, slows down the development rate of the population.

Furthermore, our results highlighted where to focus further biological investigations to achieve accurate model predictions. Additional field work and experiments are necessary to better infer the relationship between adult mortality and temperature, as well as differences between temperatures from weather stations and temperatures in tsetse fly resting places.

Conclusion: The design of control and eradication programmes requires accurate knowledge of the ecological characteristics of tsetse flies and the geographic structure of their populations. Our model includes all the features needed to provide such knowledge and can therefore support decision making.



Abstract ID 127

Selection of the Best *Glossina* Strain Suited to Local Environmental Conditions of the Target Eradication Area

Soumaïla PAGABELEGUEM[1], Sophie RAVEL[2], A. Hamadou DICKO[3], Marc J.B. VREYSEN[4], Andrew PARKER[4], Peter TAKAC[5], Karine HUBER[3], Issa SIDIBÉ[1], Geoffrey GIMONNEAU[3] and Jeremy BOUYER[4]

[1]IBD-CETT, Bobo-Dioulasso, Burkina Faso, Email: *pagasoum@yahoo.fr*
[2]Institut de Recherche pour le Développement (IRD)
[3]French Agricultural Research Centre for International Development (CIRAD), Montpellier, France
[4]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria
[5]Institute of Zoology, Section of Molecular and Applied Zoology, Slovak Academy of Sciences (SAS), Bratislava, Slovakia

Background: Tsetse flies occur in much of sub-Saharan Africa where they are vectors of trypanosomes that cause human and animal African trypanosomosis. The sterile insect technique (SIT) is currently used to eliminate tsetse fly populations in an area-wide integrated pest management (AW-IPM) context in areas of Senegal and Ethiopia.

Three *Glossina palpalis gambiensis* strains [originating from Burkina Faso (BKF), Senegal (SEN) and an introgressed strain (SENbkf)] were established and are now available for use in future AW-IPM programmes against trypanosomes in West Africa. For each strain, knowledge of the environmental survival thresholds is essential to determine which of these strains is best suited to a particular environment or ecosystem, and can therefore be used effectively in SIT programmes.

Methodology: In this study, we investigated the survival and fecundity of three *G. p. gambiensis* strains maintained under various conditions: 25 °C and 40, 50, 60, and 75 % relative humidity (RH), 30 °C and 60 % RH and 35 °C and 60 % RH.

Results: The survival of the three strains was dependent on temperature only, and it was unaffected by changing humidity within the tested range. The BKF strain survived temperatures above its optimum better than the SEN strain. The SENbkf showed intermediate resistance to high temperatures.

Conclusion: A temperature of about 32 °C was the limit for survival for all strains. A RH ranging from 40 to 76 % had no effect on fecundity at 25–26 °C. We discuss the implications of these results on tsetse SIT-based control programmes.

Key words: Tsetse flies, area-wide integrated pest management, sterile insect technique, mass-rearing, survival, fecundity, environmental conditions

Abstract ID 131

The Effect of Insecticide Impregnated Livestock Protective Fence of Selected Farmers in Three Kebeles of Arba Minch, Zuria and West Abaya District, Gammo Goffa, SNNPRS, Ethiopia

Firew Lejebo LEKA

National Institute for the Control and Eradication of Tsetse and Trypanosomosis (NICETT), Arba Minch, Ethiopia, Email: *firewye@gmail.com*

This experimental study was under taken from August, 2014 to February, 2015 in Elgo and Shara kebeles of Arba Minch Zuria, and Faragosa kebeles of Merab Abaya districts of Gamo Goffa Zone, SNNPRS (Southern Nations, Nationalities, and Peoples' Region State). For this study insecticide impregnated net was fenced around the pen of ninety selected farmers, and unfenced for fifteen farmers in the control group.

The apparent density of tsetse was reduced during the 2nd and the 3rd cycle in Elgo kebeles from 0.12 to 0.1, and from 0.01 to 0 in Shara kebeles and it was absent in Faragosa. However, the density was zero during the baseline survey, and appeared in the 2nd and 3rd cycle due to dramatic change of the season. On the other hand, densities of tsetse flies were zero in all of the three cycles in Faragosa. Biting flies were reduced from 34 to 3 in Elgo, 16 to 5 in Shara, and 19 to 1 in Fargosa kebeles during the 1st, 2nd and 3rdcycle of monitoring respectively.

From 191 animals examined for the *Trypanosoma* spp. parasite in three monitoring cycles, none of them was found positive with parasites, but the mean PCV value (packed red cell volume percent) was increased from 20.26 to 27.38 in Elgo, 17.8 to 26 in Shara, and 25.7 to 27.62 in Faragosa.

The average milk production was increased from 0.68 to 0.83 in Elgo, and 1.31 to 1.5 in Faragosa, but it was reduced from 3.1 to 1.17 in Shara due to inadequate feed provided by farmers. In addition the association between PCV and animal coat colour was checked in three of the monitoring cycles, based on this the mean PCV value was low in black and high in white followed by red animals.

On the other side the defensive movement of the animals was recorded for five minutes and it was found that the average defensive movement was reduced from 15 to 8 in Elgo, 11 to 5 in Shara and 21 to 5.5 in Fargosa kebeles in three of the cycles.

On the other hand, in the control group of fifteen farmers there was reduction in milk production, PCV value, weight gain, and increment in fly population and defensive movement.

Conclusion: From the study it was concluded that fly population, parasite and defensive movement was reduced, and milk and meat production increased.

Abstract ID 164

Quality Control of Sterile Males Provided in the Form of Irradiated Pupae to the Regional Projects of the Tsetse Eradication

Souleymane DIALLO[1], Momar Talla SECK[2], Jean-Baptiste RAYAISSE[1], Assane Gueye FALL[2], Mireille BASSENE[2], Baba SALL[3], Marc J.B. VREYSEN[4], Peter TAKAC[5], Andrew PARKER[4], Jérémy BOUYER[4] and Geoffrey GIMONNEAU[6,2]

[1]Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide, Bobo-Dioulasso, Burkina Faso, Email: *jbrayaisse@hotmail.com*

[2]Institut Sénégalais de Recherches Agricoles, Laboratoire National d'Elevage et de Recherches Vétérinaires, Dakar, Sénégal

[3]Direction des Services Vétérinaires, Dakar, Sénégal

[4]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Austria, Vienna

[5]Institute of Zoology, Section of Molecular and Applied Zoology, Slovak Academy of Sciences, Bratislava, Slovakia

[6]French Agricultural Research Centre for International Development (CIRAD), Montpellier, France

Background: Quality control studies were conducted to determinate the impact of various treatment conditions on the percentage of emergence and the quality of sterile males (percentage of flyers), by comparing different batches of pupae at the Centre International de Recherche and Développement sur l'Elevage en zone Subhumide (CIRDES / Bobo – Dioulasso, Burkina Faso).

Methodology: The following treatments were compared: A0 = control group of 50 pupae (equivalent to the conditions of the colony); A1 = group of 50 pupae after chilling (10°C); A2 = group of 50 pupae after chilling & irradiation; A3 = group of 50 pupae after chilling & irradiation & second chilling after irradiation; and A4 = pupae with all previous treatments plus the transport by air from CIRDES to Dakar. In addition, flies emergence rate from the Slovakia Academy of Sciences (SAS) were evaluated for the A1 treatment.

Data were analysed using binomial mixed effects models using the emergence rate or percentage of flyers as the response variables, the batch origin and treatment type as fixed effects and the date of arrival at Institut Sénégalais de Recherche Agricole (ISRA/Dakar) as a random effect.

Results: The model showed for flies from CIRDES, that the first and second chilling (A1 and A3) rounds had a significant negative impact on the emergence rate ($p<10-3$) in comparison to A0, whereas irradiation (A2) did not reduce it further ($p>0.05$). The emergence rate was also superior for A1 at CIRDES than at SAS ($p<10-3$), although the mean values were very close.

Considering the rate of operational flies, the first chilling and the irradiation both reduced the quality of the flies at CIRDES ($p<10^{-3}$, the reference level is CIRDES_A1 in the model presented upon). At stage A1, the rate of operational flies was better in SAS than CIRDES ($p<10^{-3}$). Interestingly, the second chilling (A3) event allowed a significant "recovering" of the rate of operational flies at CIRDES ($p<10^{-3}$, the reference level was set to CIRDES_A2 to calculate this probability).

Finally, the transport from CIRDES (A4) to Dakar also significantly reduced the quality of the sterile males ($p<10^{-3}$, the reference level was set to CIRDES_A3 to calculate this probability). This was probably due to physical injuries during transport.

Key words: Sterile males, pupae, quality control, chilling, irradiation, transport

Abstract ID 183

A Staged, Progressive Pathway for the Control and Elimination of Tsetse-transmitted African Animal Trypanosomosis

Giuliano CECCHI[1], Oumar DIALL[1], Gift WANDA[2], Rafael ARGILÉS-HERRERO[3], Marc J.B. VREYSEN[3], Giovanni CATTOLI[3], Gerrit VILJOEN[3], Raffaele MATTIOLI[1] and Jérémy BOUYER[3]

[1]Food and Agriculture Organization of the United Nations (FAO), Sub-regional Office for Eastern Africa, Addis Ababa, Ethiopia, Email: *giuliano.cecchi@fao.org*
[2]PATTEC, African Union, Addis Ababa, Ethiopia
[3]Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria

Background: Progressive Control Pathways (PCP) and the related implementation roadmaps are used in the control and elimination of a number of human and animal diseases, including foot-and-mouth disease, peste des petits ruminants, brucellosis and rabies. International organizations such as FAO, OIE and WHO, rely on PCP frameworks for planning, implementing and evaluating interventions against diseases. Flexible, stepwise PCPs enable to structure the road to disease freedom through a series of achievable, discrete stages. Here we outline a novel PCP for the control and elimination of tsetse-transmitted African animal trypanosomosis (AAT), the bane of poor livestock keepers in sub-Saharan Africa.

Methodology/Results: The PCP for AAT is structured along five stages and a pre-entry level, i.e. 'below Stage 1' (Figure). A regular step-wise progression is the rule (i.e. from Stage N to Stage N + 1) but fast-tracking is possible in specific circumstances. In order to move from one stage to the next, the set goals for the ongoing stage must have been achieved, and a plan for the following stage must be prepared. Independent validation is required.

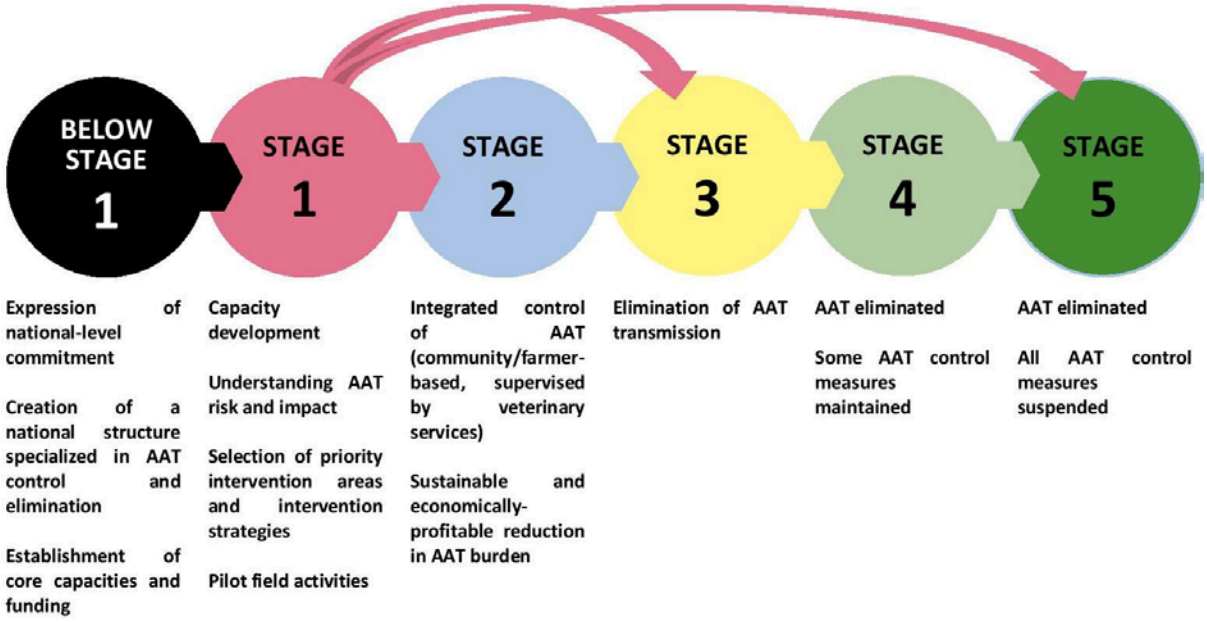


Fig. 1. *Progressive pathway for the control and elimination of AAT.*

Key requirements for a country to enter the PCP (i.e. to move to 'Stage 1') include political and financial commitment for the progressive control and elimination of AAT, and the existence of a functioning Specialized National Structure having core capacities and mandate to deal with tsetse and AAT. When in Stage 1, affected countries have to develop technical capacities, and gain a sufficient understanding of AAT distribution, risk and impact for an evidence-based planning of subsequent activities; pilot field interventions are also conducted.

Larger scale field activities are implemented in Stage 2 and beyond, within the priority areas identified in Stage 1. Stage 2 aims at a sustainable reduction of the AAT burden, and the intervention strategy hinges on the integrated control of AAT (a community/farmer based approach).

The focus of the PCP's final stages (3 to 5) is to create sustainable AAT-free areas. Stage 3 is completed when AAT transmission is interrupted. In Stage 4, some control measures are maintained, while in Stage 5 the elimination of AAT must be sustainable in the absence of interventions.

Conclusions: AAT elimination normally requires the elimination of the tsetse vector. The sterile insect technique (SIT), which works best when wild tsetse densities are very low, can be a useful tool in the elimination scenario. The PCP for AAT is consistent with the phased conditional approach recommended by FAO/IAEA for the implementation of area-wide integrated pest management programmes that might include the SIT. Importantly, the PCP enables to better position tsetse and AAT interventions in the broader context of poverty reduction, hunger eradication and increased resilience of vulnerable and marginalized rural communities, which are some of the major objectives of FAO and are included in the Sustainable Development Goals.

Abstract ID 207

Spatial Distribution and Trypanosome Infection of Tsetse Flies in the Sleeping Sickness Focus of Zimbabwe in the Hurungwe District

William SHERENI[1], Neil E. ANDERSON[2], Learnmore NYAKUPINDA[1] and Giuliano CECCHI[3]

[1]Tsetse Control Division, Department of Livestock and Veterinary Services, Ministry of Agriculture, Mechanization and Irrigation Development, Harare, Zimbabwe, Email: *shereni2005@yahoo.com*
[2]The Royal (Dick) School of Veterinary Studies and the Roslin Institute, University of Edinburgh, Roslin, UK
[3]Food and Agriculture Organization of the United Nations (FAO), Sub-regional Office for Eastern Africa, Addis Ababa, Ethiopia

Background: In Zimbabwe, cases of human African trypanosomiasis (HAT) are caused by the unicellular protozoan *Trypanosoma brucei*, sub-species *T. b. rhodesiense*. They are reported from the tsetse-infested area in the northern part of the country, broadly corresponding to the valley of the Zambezi River. Tsetse-transmitted trypanosomes, in particular *T. congolense* and *T. vivax*, also cause morbidity and mortality in livestock, thus generating poverty and food insecurity.

Two species of tsetse fly, *Glossina morsistans morsitans* and *G. pallidipes*, are known to be present in the Zambezi Valley, although their distributional patterns and densities have not been investigated in detail. The present study tries to address this gap as well effects of habitat fragmentation on the dynamics of trypanosomiasis in humans and livestock. The study identified areas where tsetse control interventions, including suppression activities and sterile insect releases, should be focused to achieve the greatest impact.

Methods: Tsetse distribution and trypanosome infections were studied using traps and fixed fly rounds located at 10 km intervals along a 110 km long transect straddling the southern escarpment of the Zambezi Valley. Three km long fly rounds were conducted on 12 sites, and were repeated 11 times over a 7-month period. Additional traps were deployed and monitored in selected sites. Normalized difference vegetation index (NDVI) was used as a measure of vegetation cover. Microscopic examination of 2092 flies for trypanosome infections was conducted.

Results: Surveys confirmed the presence of *G. morsitans morsitans* and *G. pallidipes* in the Zambezi Valley floor. Moving south, the apparent density of tsetse flies appears to peak in the vicinity of the escarpment, then drops on the highlands. Only one fly was caught south of the old game fence separating protected and settled areas in a drainage area where the vegetation cover was relatively denser. A trypanosome infection rate of 6.31% was recorded in tsetse flies dissected. Only one infection of the T. *brucei*-type was detected.

Conclusions: Tsetse fly distribution in the study area appears to be driven by ecological factors such as variation in land use and habitat fragmentation, and altitude-mediated climatic patterns. Targeted control of tsetse flies is recommended in areas of heterogeneous vegetation patterns. Trypanosome infections in tsetse flies are consistent with HAT epidemiological data, which categorize the situation to be generally 'low risk'.

Key words: Tsetse, Glossina*, sleeping sickness, African animal trypanosomiasis, Zimbabwe, epidemiology*

Abstract ID 208

Buffalo Flies (*Haematobia irritans exigua*) Expanding their Range in Australia: The Opportunity for Area-wide Controls

Peter JAMES[1], Mukund MADHAV[1] and Geoff BROWN[2]

[1]University of Queensland, St. Lucia, Australia, Email: *p.james1@uq.edu.au*
[2]Department of Agriculture and Fisheries, Queensland

Background: Buffalo flies (BF) (*Haematobia irritans exigua*) and the very closely related horn flies (*Haematobia irritans irritans*) (HF) have proven to be extremely invasive species. Both have significant economic and welfare impacts on beef and dairy production. Horn flies entered the USA from Europe in 1886 and rapidly spread through cattle production areas in most of North and South America. Buffalo flies entered Australia in 1838 and spread relatively slowly to reach Bundaberg (24.8^0S latitude) in eastern Australia by 1946. No further spread was recorded for the next 30 years. However following mild seasons in the late 1970s, southward expansion of BF range resumed and appears to have accelerated in recent years.

The distributions of HF and BF intersect in South East Asia with HF present in Mongolia, northern China and Japan and BF in India, Taiwan, SE Asia, New Guinea, and northern Australia. Although HF spread rapidly through the tropical areas of Central and South America, similar spread has not occurred in Asia.

Methods: Recent records and reports of the occurrence of BF in Australia were collated and mapped. CLIMEX modelling was conducted to determine potential spread of BF under predicted climate change. Buffalo flies collected from throughout their range in Australia and from Bali in Indonesia were tested to for the presence of *Wolbachia*.

Results: Buffalo flies have expanded their range 1000 km southward (to 32.7^0S) in the last 40 years. CLIMEX modelling suggests that climate change will facilitate further rapid spread into new areas and result in increased economic and welfare impacts in areas where BF is already present. The model predicted the potential establishment of persisting BF populations as far south as South Australia and southern Western Australia by 2030.

The preponderance of susceptible *Bos taurus* type cattle, the absence of need for other parasite control treatments through most of Australia's southern cattle industries and the development of chemical resistance in BF populations will facilitate this spread.

Whereas *Wolbachia* appears to be ubiquitous in HF, it was not found in BF collected from Australia or Indonesia. This may be a factor contributing to the apparent failure of HF and BF to interbreed and of BF to spread southward in SE Asia.

Conclusions: Without intervention, BF are likely to become major cattle pests in Australia's southern beef and dairy industries. Reliance on chemical treatments and regulated cattle movements have previously proven unsuccessful in preventing spread.

In contrast to HF, BF does not have a pupal overwintering phase but survives winter in localised foci of low populations of slowly cycling flies. Populations increase and spread to infest surrounding areas when weather becomes favourable in summer. This suggests the potential for area-wide approaches to preventing range expansion and reducing impacts in endemic areas.

We have now commenced a project to transfect BF with *Wolbachia*, to assess the effects of *Wolbachia* infection and to determine the feasibility of control by directly targeting overwintering foci of BF.

Abstract ID 214

Microbiota Diversity in *G. pallidipes* from Three Locations in Tanzania as Revealed by High Throughput Sequencing and their Implication for Tsetse Refractoriness

Imna MALELE[1], Eugen LYARUU[1], Anne GEIGER[2] and Hamis NYINGILILI[1]

[1]Vector & Vector Borne Diseases Institute, Tanzania Veterinary Laboratory Agency (TVLA), Tanga, United Republic of Tanzania, Email: *maleleimna@gmail.com*
[2]UMR 177, IRD-CIRAD, Montpellier, France

Background: Studies of microorganisms living in insect guts have increased considerably ranging from diversity screening using metagenomic surveys to molecular studies of the interaction of gut bacteria with the host immune system and the response to infection. Microbes associated with eukaryotic organisms are known as microbiota. Microbiotas found in the gut play a crucial role in the host physiology (for example digestion of nutrients and regulation of immune responses). In insect vectors, for instance tsetse, microbiotas have been implicated to influence the establishment of parasites in the gut in the transmission dynamics of trypanosomiasis and vector competence.

Methodology: High throughput sequencing of midgut bacteria from *Glossina pallidipes* from three locations: Serengeti (a sleeping sickness focus) and Kabuku and Morogoro (non-sleeping sickness areas) were conducted by analysing a total of 35 midguts. The total read counts per bacteria were log transformed to standardize the data.

Results: Results showed five major phyla namely Proteobacteria, uncultured bacterium, Firmicutes, Bacteroidetes and Acidobacteria. The Proteobacteria were dominant across all *G. pallidipes* from all locations, but the uncultured and Firmicutes groups were dominant in *G. pallidipes* Kabuku. When comparing diversity at Family level, Enterobacteriaceaea (82%), uncultured (68%) and Bacillaceae (8%) were dominant in *G. pallidipes* Kabuku whereby in other locations the prevalence was below 10%. The trend was observed to the level of order classification.

The genus level showed a rich diversity of different bacteria in *G. pallidipes* Serengeti compared to *G. pallidipes* from Kabuku and Morogoro as presented in Figure 1. The exceptional was the occurrence of *Wigglesworthia glossinidia* (3.3%) and secondary endosymbiont (1.2%) in *G. pallidipes* Kabuku and non in *G. pallidipes* Morogoro and *G. pallidipes* Serengeti. Unidentified bacteria were found in *G. pallidipes* from Morogoro and Kabuku. The trypanosome infection in *G. pallidipes* Serengeti was nil, 40% in *G. pallidipes* Morорgoro and 47% in *G. pallidipes* Kabuku.

Conclusion: The occurrences of different bacteria from the guts of tsetse flies reveals a rich diversity of microbes and calls for comprehensive studies to establish their role in a blood

feeding insect and their implication in the dynamics of African trypanosomiasis and tsetse refractoriness.

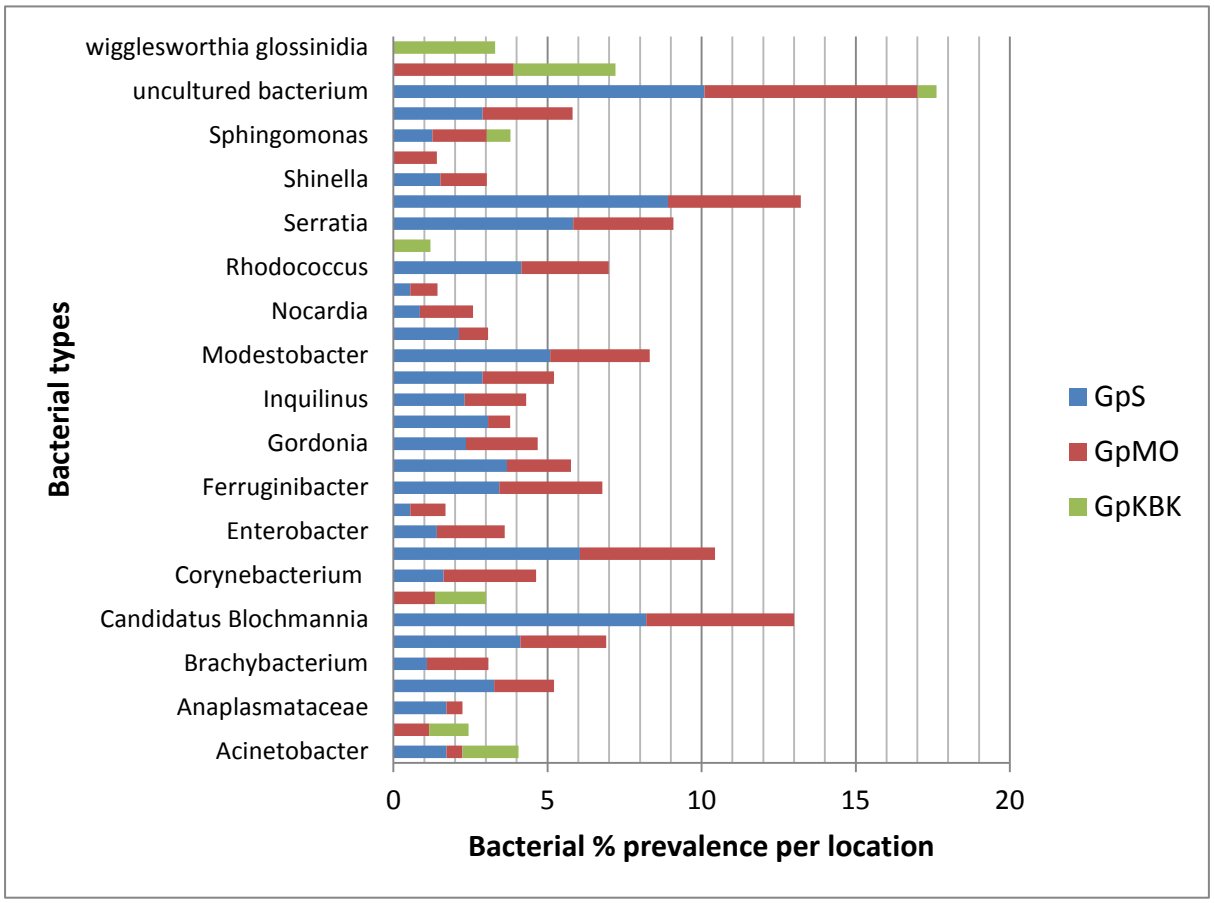


Fig. 1*: Diversity of bacteria from* G. pallidipes *from three locations in Tanzania.*

Abstract ID 217

Populational Study of the New World Screwworm Fly in Peru, *Cochliomyia hominivorax* (Diptera: Calliphoridae)

Luana BERGAMO[1], Pablo FRESIA[2], Robin MADUEÑO[3] and Ana Maria AZEREDO-ESPIN[1]

[1]Universidade Estadual de Campinas, UNICAMP, São Paulo, Brazil, Email: *amlazeredo@gmail.com*
[2]Instituto Pasteur, Montevideo, Uruguay
[3]Dirección de Sanidad Animal, SENASA, Perú

Background: Livestock production is an important economic activity in South America, but has been suffering significant losses due to the impact of parasites. The New World screwworm fly (NWS), *Cochliomyia hominivorax*, is an important ectoparasite and myiasis-causing fly endemic from the Americas. The current geographic distribution of NWS fly is Neotropical, having been eradicated in North and Central America after the implementation of an area-wide programme integrating the Sterile Insect Technique (SIT).

Planning a similar area-wide control programme in South America could be an interesting alternative to the exclusive use of insecticides, but requires first the delineation of populations, regions and geographic scales. Studies that investigated the genetic structure of NWS fly populations and dispersal corridors for the species indicated that the distribution pattern of the species is complex.

Considering this scenario, the present work investigates the genetic variability and population structure of *C. hominivorax* samples from Peru, an unexplored country from South America that seems to be an important region for the dispersion of the species and connection between distinct areas.

Methodology: Samples from different regions from Peru, encompassing all the territory limits, were collected from April to June 2016 in collaboration with SENASA-Peru. These samples are being analysed with three mitochondrial DNA regions (COI, COII and CR) and here we present preliminary results obtained from COI and COII mitochondrial regions sequences for 65 individuals.

Results: AMOVA and pairwise FST results suggest the presence of four genetically distinct groups with some geographic restriction. Two of these groups, called Loreto and Moquegua according to their geographic location, present exclusive haplotypes. The 10 sampling locations considered presented high haplotype diversity, low nucleotide diversity and Tajima's D and Fu's Fs significant negative values, indicating a probable demographic process of population expansion.

Conclusion: As mentioned above these results are just preliminary and we will include more samples and other mitochondrial marker (CR) to conduct population diversity and structure

analyses again. The data obtained from Peru will also be included in a big dataset from our laboratory, which includes mitochondrial sequences from all *C. hominivorax* samples analysed since 2011, and a spatial analysis will be done with the aim to corroborate the presence of a corridor trough this region of South America.

Key words: Demographic process, dispersion corridors, mitochondrial DNA, population structure, spatial analysis

Abstract ID 235

African Animal Trypanosomosis (AAT) in the Kadiolo Circle: Entomological and Parasitological Survey Results

Modibo DIARRA[1], Zakaria BCOUM[2], Hamidou M. MAIGA[3] and Youssouf SANOGO[2]

[1]Project for the Control of Tsetse Flies and Animal Trypanosomoses (P.L.M.T), Sikasso, Mali, Email: *modibododo@yahoo.fr*
[2]Central Veterinary Laboratory (CVL), Bamako, Mali
[3]University of Sciences, Techniques and Technology, Bamako (USTTB), Mali

Background: In the late 2000s, significant mortalities of cattle were reported in the Kadiolo Circle following an explosion of African animal trypanosomiasis (AAT). Disease and mortality have directly affected agricultural production and the savings of agro-pastoralists. In 2008, the analysis of the blood samples taken from the Circle by the Central Veterinary Laboratory of Bamako confirmed the presence of trypanosomiasis with parasitic prevalence between 25% and 64.7%. This cross-sectional survey will help plan an IPM strategy that is effective and environmentally appropriate.

Methodology: Parameters such as the parasite prevalence of trypanosomes and the apparent density (DAP) of tsetse flies have been studied in the Kadiolo Circle, which occupies the southern part of the Sikasso region in southern Mali, where the risk of animal trypanosomosis is high throughout the year. For DAP surveys, 99 catch-baited Challier-Laveissière traps were distributed among 28 villages in the municipalities of Misséni, Fourou, Kadiolo, Zégoua, Dioumatènè and Diou.

For each locality the species of tsetse caught and the bulk density were determined. In all, a sample of 404 bovines of different sexes, breeds and ages from herds from 14 villages was randomly assigned (30 bovines / village). Blood was harvested from the animals and submitted to the Buffy Coat Technic Diagnostic. Trypanosome species, trypanosomiasis prevalence and hematocrit value in village farms were determined.

Results: Glossina palpalis gambiensis, *Glossina tachinoides* and *Glossina morsitans submorsitans* were the species observed. The average tsetse DAP on the points examined was equal to 6.34 tsetse / trap / day. The most infested communes were: Dioumatenè, Fourou, Misséni, Kadiolo and Zégoua with respective DAPs equal to 14.4, 7.32, 7.18, 5.16 and 4 tsetse / trap / day.

Of 404 cattle from which blood was harvested and submitted to Buffy Coat Technique diagnosis, 60 positive cases were observed with a prevalence of 14.85%. The highest rates were recorded in the villages of Dioumatènè (50%), Kafono (43.33%), Fanidiama (22.5%), N'Golopénè (30%), Lofigué (20%) and Borogoba (20%). The mean value of the hematocrit was 26%. Cattle were mainly infected with *Trypanosoma congolense* and *Trypanosoma vivax*.

Conclusion: Based on survey results, a significant decrease in tsetse DAP and the prevalence of AAT was perceptible in the Circle compared to previous data. This decrease would be due to the change of landscape observable on the ground as a result of human action, climate change and the dilution of trypanotolerant breeds. The control strategy integrating chemotherapy, vector control and breeding of trypanotolerant breeds remains the best approach to be preferred in the Circle of Kadiolo.

Abstract ID 251

Analysis the Impact of Irradiation Treatment on the Establishment of *Sodalis* in *Glossina morsistans morsitans* Species

Guler DEMIRBAS UZEL[1,2], Andrew PARKER[1], Marc VREYSEN[1], Robert L. MACH[2], Kostas BOURTZIS[1] and Adly ABD-ALLA[1]

[1]Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, Email: *G.Demirbas-Uzel@iaea.org*
[2]Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria

Background: Tsetse flies (Diptera: Glossinidae) are the sole vectors of African trypanosomosis, the cause of sleeping sickness disease in humans and nagana in animals. Similar to most eukaryotic organisms, *Glossina* species have constituted symbiotic relation with microorganisms, i.e. bacteria. Three main symbiotic bacteria have been found in tsetse flies are *Wigglesworthia glossinidia*, an obligate symbiotic bacterium, the secondary endosymbiont *Sodalis glossinidius*, and the third is the reproductive symbiont *Wolbachia pipientis*. In addition to the symbiont bacteria, the salivary gland hypertrophy virus which modulates the fecundity of the infected flies was reported in several tsetse species.

Due to the lack of effective vaccines and cheap drugs for trypanosomes, and the development of resistance of the trypanosomes against the available trypanocidal drugs, vector control remains the most effective approach for sustainable management of these diseases.

Among the control methods used for tsetse flies, the Sterile Insect Technique (SIT) is an effective method to suppress and/or eradicate tsetse flies. The SIT is based on the mass-production of the targeted insect, separation and irradiation of males, and their release into the natural habitat. Sterile males are routinely fed trypanocides before release to minimize any possibilities of disease transmission.

Methodology: To further avoid any potential of disease transmission during SIT application in an tsetse endemic area with human sleeping sickness, symbiont-based approaches are being investigated to produce tsetse males that are fully refractory to trypanosome infection. To do so, modified symbiont bacteria (*Sodalis*) producing anti-trypanosomes proteins need to be maintained in the males, which will then be treated with sterilizing irradiation treatment and released. The impact of the irradiation treatment on the establishment of *Sodalis* bacteria was investigated, as it will largely determine the feasibility of this approach.

Results: The results indicate that the irradiation treatment (110 Gy) applied to 5-7 days old *Glossina morsitans morsitans (G.m.m)* flies did not cause visible effects on the mutation rate (SNPs (Single Nucleotide Polymorphism)) as tested in 15 genes distributed over the *Sodalis* genome. However, the irradiation treatment had a negative impact on the multiplication of *Sodalis* and salivary gland hypertrophy virus compared to non-irradiated flies. On the other

hand, the irradiation treatment increased the *Wolbachia* prevalence compared to the non-irradiated control.

Conclusion: Taken together, these results indicate that the irradiation treatment has a significant impact on tsetse symbionts and pathogen, and might represent a challenge for the combination of the para-transgenesis approach in the frame of SIT programmes to control tsetse flies.

Key words: Symbiosis, genomic microbiology, SNP

Abstract ID 253

Identification of Cultivable Tsetse Gut Microbiota and Assessment of Their Probiotic Potential to Improve Fly Quality and Performance for the SIT

Henry KARIITHI[2], Irene K. Meki[1,3], Marc J. B. VREYSEN[1], Andrew G. PARKER[1], Kostas BOURTZIS[1] and Adly M. M. ABD-ALLA[1]

[1]Insect Pest Control Laboratory, Seibersdorf, FAO/IAEA, Austria, Email: *H.Kariithi@iaea.org*
[2]Biotechnology Research Institute, Kenya Agricultural and Livestock Research Organization, Kenya
[3]Laboratory of Virology, Wageningen University, The Netherlands

The control of tsetse vectors via the sterile insect technique (SIT) involves mass-production of sexually sterile males. However, the irradiation procedure used to induce male sexual sterility potentially damages the gut epithelia and the beneficial gut-inhabiting microbiota. These effects may reduce the quality (sexual performance and competitiveness) of the sterile males, thereby negatively affecting the success and operational costs of SIT. It is therefore paramount to enhance the quality of the sterile males. A potential strategy to achieve this is to exploit the beneficial traits conferred by the gut microbiota to their insect hosts.

We used culture-dependent and culture-independent methods to determine the variability of tsetse gut microbiota, identify which of the bacterial species are cultivable, and assess the amelioration potential of a selection of the cultivable species to improve the quality of sterile males. Both methods were based on PCR-amplification of the bacterial 16S rRNA gene. The assessment of microbiota diversity and abundances was performed at different fly developmental stages (larvae, pupae and adults), and on flies of different ages that emerged from irradiated pupae. Probiotic potential of the bacteria was assessed by offering experimental flies with blood meals supplemented with a selection of candidate bacterial isolates.

Notable of the identified gut microbiota were members of family Microbacteriaceae (genus *Microbacterium*), Sphingobacteriaceae (genus *Sphingobacterium*) and Moraxellaceae (genus *Acinetobacter*). These bacterial species have been reported in other insects, some of which are documented to confer beneficial traits in their insect hosts. These results indicate that the densities of the cultivable gut microbiota significantly decrease in 6 to 10 days old flies; this age is important for the release of SIT sterile males into the wild populations for vector control. Secondly, the gut microbiota community was composed of both beneficial and potentially harmful bacterial species. Consequently, in addition to exploiting the beneficial bacteria, it may be necessary to design a strategy to reduce the populations of the harmful bacterial species.

In terms of SIT applications, the identified *S. marcescens* (with anti-parasitic benefits) can be targeted to enhance anti-trypanosome capabilities of SIT sterile males.

Abstract ID 261

Studies of the Impact of Myiasis in Uruguay: Survey of Occurrence in the Department of Artigas during the Winter 2015

Martin ALTUNA, Maria Victoria IRIARTE and Laura MARQUES

Ministerio de Ganaderia Agricultura y Pesca, Artigas, Uruguay, Email: *maltuna@mgap.gub.uy*

In Uruguay the economic impact associated with the occurrence of myiasis in production animals and some aspects related to the epidemiology of the parasite have been studied through epidemiological data surveys. In addition, a sampling and identification of larvae of myiasis cases registered was performed in the department of Artigas.

According to the results, of the national survey conducted in 2014, cases of myiasis occur throughout the country with decreases during the winter. The 8.4% of the ovine are affected, with the sheep being the most affected category, and 3.7% of the cattle, the calves being the predominant category. Mortality associated with myiasis is 2.1% in ovine and 0.1% in cattle.

In the department of Artigas, the most frequent location of myiasis in cattle is the navel (42%), while in ovines the hooves (68%). The field activities most likely to end in myiasis are castration, identification with earrings, iron branding in cattle, while in ovines they are shearing, sheep mating and castration. Out of a total of 164 farmers, 41% answered that they perceived a decrease in the effectiveness of some products used for myiasis treatment, and 15% have stopped using some of those products because of this.

Monitoring of myiasis with sampling and identification of larvae, in 9 farms in the department of Artigas, carried out by veterinarians during the winter of 2015, registered 103 cases. During the 13 weeks covered by the study, in 11 weeks cases of myiasis (84.6%) were recorded. In 98% of cases New World screwworm was identified when processing more than 650 larvae.

The following table summarizes the use of specific veterinary products by veterinarians according to their forms of presentation (spray, injectables, ointment, liquid and dust) in the department of Artigas:

Table 1. *Use of products for treatment of myiasis*

Species	1 product	2 products	3 products	4 products	5 products
Cattle	41%	41%	13%	3%	1%
Ovine	40%	39%	16%	4%	1%

The active ingredients most used in cattle and ovines are the combination of phosphorus and pyrethroid, and to a lesser extent macrocyclic lactones and fipronil.

The results of the studies show the importance of myiasis in the livestock sector, causing annual losses of hundreds of millions of dollars. It is also worth mentioning that, as a zoonosis, cases are recorded in humans, which according to projections from a study carried out in 2006, would reach 818 per year, mainly in the rural population.

In view of this reality, it is necessary to continue the epidemiological studies at the field level, to deepen the research regarding the sensitivity and effectiveness of the active principles used in the treatments and prevention of myiasis. There is also the need to carry out campaigns to raise awareness of the problem on a large scale and training in the area of good farming practices.

Finally, it is hoped to establish a regional programme for the control and subsequent eradication of New World Screwworm, which will undoubtedly bring benefits to the economies of the countries and human health.

Abstract ID 274

Mass-Rearing of *Glossina fuscipes fuscipes* in the Kality Insectary, Addis Ababa, Ethiopia

Kumela Lelisa DERA

National Institute for the Control and Eradication of Tsetse Fly and Trypanosomosis, Addis Ababa, Ethiopia, Email: *lelisakumela@gmail.com*

The National Institute for the Control and Eradication of Tsetse and Trypanosomosis (NICETT) is currently releasing sterile males as the final component of an AW-IPM project for the elimination of the *G. f. fuscipes* in the Deme Valley of Ethiopia. These sterile males are produced in the Kality Tsetse Fly Mass-Rearing and Irradiation Center, Addis Ababa, Ethiopia.

In the last 18 months (from September 2015 to January 2017), the performance of the colony has reversed its sustained declining trend, from an average weekly growth rate of -2.09% in the interval between week 42-2015 and week 8-2016, to an average growth rate of +2.99% in the interval between week 42-2015 and week 8-2016. The pupae production and the colony size have tripled during this interval.

The suggested reasons for this change in colony performance are the strict adherence to the standard operation procedures and the improvement in the management of the insectary. Among the first, those who have played a major role are: i) the number of feeding rounds per membrane has been reduced, ii) an alternate feeding calendar has been adopted, iii) the maintenance of environmental conditions within the optimal range has improved, iv) the settings of the feeding mats have been adjusted to optimal temperature of the blood, v) the quality of the blood used for feeding has improved and is strictly assessed before being used, vi) the sex ratio in the cages has been optimised, and vi) the pupae emergence room has been separated from the pupae maturation room.

Regarding the management of the insectary, the implementation of a networked database system has significantly improved the data collection, storage and analysis. The commitment of the rearing technicians has increased thanks to the implementation of a permanent training programme, thus reducing the turnover rate.

Key words: *Glossina fuscipes*, mass-rearing, Kality, Ethiopia

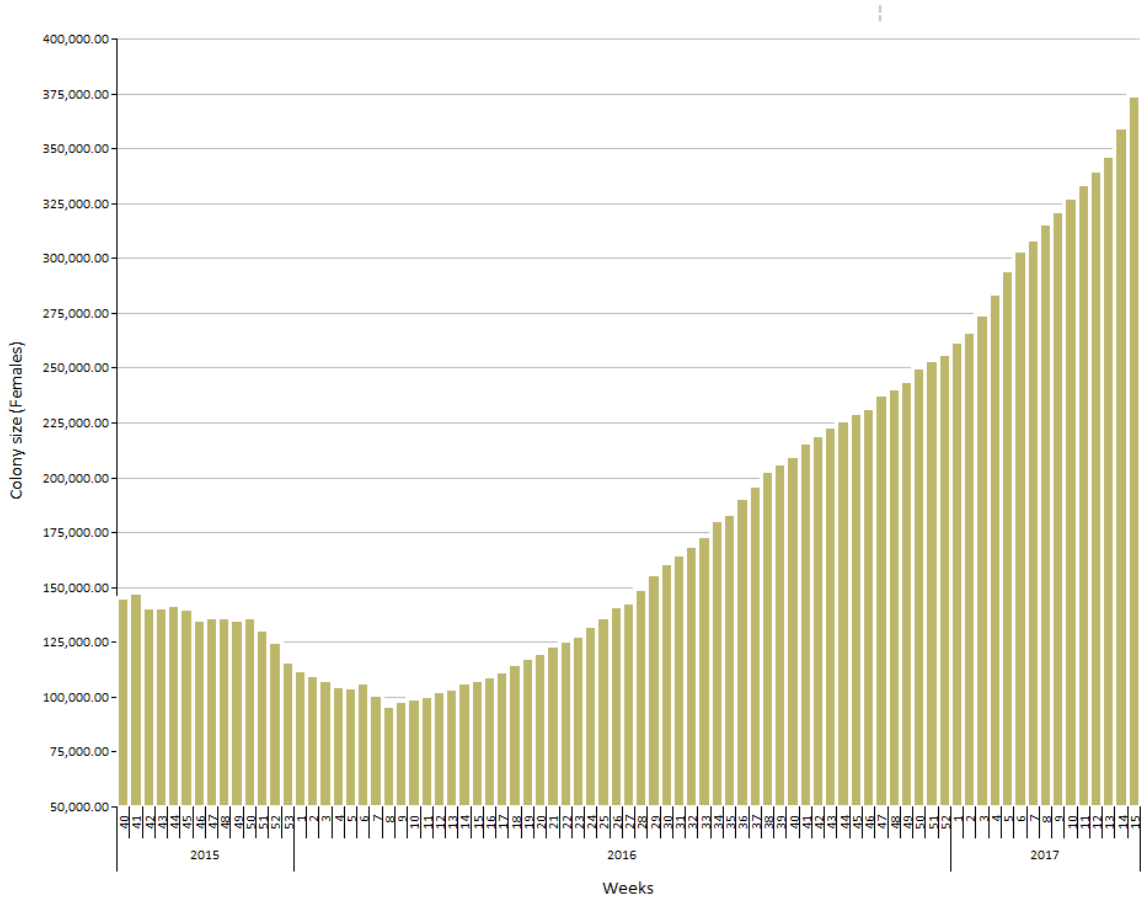


Fig. 1. *Colony size of* Gossina fuscipes fuscipes *from week 40/2015 to week 4/2017.*

Abstract ID 257

Contributing to the Resolution of Taxonomic Puzzles: Multiple Molecular Tools and Development of Protocols for the Accurate Identification of Tsetse Species

Antonis AUGUSTINOS[1], Irene MEKI[1,2], A. SARIDAKI[3], Guler DEMIRBAS UZEL[1], George TSIAMIS[3], Monique M. VAN OERS[2], Marc VREYSEN[1], Andrew PARKER[1], Adly ABD-ALLA[1] and Kostas BOURTZIS[1]

[1]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria, Email: *i.meki@iaea.org*
[2]Laboratory of Virology, Wageningen University, Wageningen, The Netherlands
[3]Department of Environmental and Natural Resources Management, University of Patras, Agrinio, Greece

Background: Tsetse flies are solely responsible for the cyclical transmission of African trypanosomes, causative agents of sleeping sickness in humans and nagana in livestock. Due to the lack of efficient and cost-effective vaccines and drugs, vector control remains the most sustainable alternative to control these diseases.

The sterile insect technique (SIT), which is a species-specific and environmentally-friendly method, has been used successfully to suppress or even eradicate populations of several *Glossina* species of economic and/or clinical importance. The development and application of the SIT package against populations of tsetse species includes laboratory domestication of a target population, mass-rearing, irradiation, handling, transport and release of sterile males in the field, accompanied by quality control analysis with an emphasis on male mating competitiveness, as well as pre- and post-release monitoring in the target area.

A key component for the successful development and implementation of the SIT package is the accurate identification of the tsetse species in the populations, both in the laboratory and in nature, which is currently largely based on time-consuming and sometimes problematic classical taxonomic approaches.

Methodology: In this study, we investigated the use of the nuclear internal transcribed spacer sequence (ITS1), microsatellite markers, and *Wolbachia* infection to distinguish tsetse species by PCR and gel electrophoresis. The sequencing and phylogenetic analysis of mitochondrial genes (COI, COII, 16S rRNA and 12S rRNA), was applied to further validate the identity of the species. Nine tsetse species maintained at the Joint FAO/IAEA Insect Pest Control Laboratory were used in this study.

Results: The results indicate that *G. pallidipes* and *G. f. fuscipes* can be identified based on sequence length variation of the ITS1. The detection of *Wolbachia* infection distinguishes *G. m. centralis* from *G. m. sub-morsitans*, and *G. m. morsitans* from *G. brevipalpis* and *G.*

swynnertoni. Finally, the use of specific microsatellite markers can distinguish *G. swynnertoni* from *G. brevipalpis*, and *G. p. gambiensis* from *G. tachnoides*. The phylogenetic analysis using mitochondrial markers confirmed the identities established with the above mentioned combination approach.

Taken together, we provide a rapid and accurate identification method for tsetse species, which is a prerequisite for the successful development and implementation of any tsetse control programme that has an SIT component.

Poster Presentations Session 4: Regulatory Issues and Socio-Economic Impact

Abstract ID 27

Bactrocera dorsalis (Diptera, Tephritidae) Status in Litchis Orchard in the Eastern Part of Madagascar

Zelin Tefiarisoa RATOVONOMENJANAHARY, Onidera ANDRIAMAMPIANINA, Voloniaina RAKOTOMANANA and Adrien RAOELIJAONA

Ministry of Agriculture, Antananarivo, Madagascar, Email: *rabeketsa@yahoo.fr*

Background: Madagascar is the fourth biggest litchi exporter in the world, with 20,000 tons annually,. The first detection for *Bactrocera dorsalis* was on December 2010 in eastern Madagascar. Due to the presence of *B. dorsalis* some countries requested establishment of areas free of this fruit fly before importing litchi fruit.

Methodology: A survey was conducted in litchi orchards in eastern Madagascar, where more orchards are under surveillance for this pest to elicit its population dynamics in order to control it. Some multilure traps were set with methyl-eugenol, a *B. dorsalis* specific male attractant. In addition, BioLure, a female-biased attractant, was placed in the orchards to attract the female fruit fly. Those attractants were used with the insecticide DDVP, and orchard sanitation was applied to prevent pest outbreaks. Some host plants were collected to detect *B. dorsalis* presence. Four litchi orchards were selected for further testing and more traps were set up inside and outside of the orchards. This survey was conducted during 3 months, from October to December 2015. A total of 63 methyl-eugenol traps and 72 BioLure traps were set up.

The capture rate of *B. dorsalis* was 0.02 flies/trap/day (FTD) in methyl-eugenol traps and 0.05 ± 0.2 FTD for BioLure traps. Approximately 3 kg of litchi fruits were collected and held, but no *B. dorsalis* flies emerged from them. The farmer dug a hole of approximately 2 m^3, placed all fruit inside and covered it with soil.

Conclusion: The presence of *B. dorsalis* in the litchi orchards is very low and no *B. dorsalis* have emerged from litchi fruit. Fly presence in the orchards originates from wild host plant or an alternative host present in the surroundings of the orchards, or sometimes infested fruits are carried for consumption from the market to an orchard where the fruit flies emerge

Abstract ID 40

Bactrocera dorsalis Survey on Mango Orchards in Western Madagascar

Irina Nantenaina RAKOTOMALALA ANDRIANAVALONA

Plant Health, Ministry of Agriculture, Antananarivo Madagascar, Email: *irinaandrianavalona@gmail.com*

Background: Mango is one of the major fruits exported from Madagascar. However, the fruit quality has not been stable because of fruit fly problems. For many years, *Ceratitis cosyra* was the main pest, but it seems that the invasive *Bactrocera dorsalis* has been displacing it in mango. To manage the pest, orchard sanitation and fly mass-trapping have been used.

Methods: Recently, a new focus has been initiated in the western region of the island to determine the population dynamics of the mango fruit flies and their infestation density in order to establish a control programme. The activity was carried out from September 2016 to January 2017, coinciding with the mango harvest season. For each orchard, 12 traps using BioLure to catch *C. cosyra* and methyl-eugenol for *B. dorsalis* were used. The surveillance was done every fortnight, the flies caught were counted and identified, and the flies / trap / day computed.

Results: The findings for the months of September and October show that *B. dorsalis* was the only species caught in traps, confirming displacement of *C. cosyra* by *B. dorsalis* in mango orchards. In this paper, we argue that even though sanitation and mass trapping systems have been used in the past to manage fruit flies in Madagascar, these cannot provide the desired control of these pests in a sustainable manner.

Conclusion: We provide detailed consideration of an area-wide integrated pest management approach for these fruit fly pests in the country that will, based on past experience, include the use of protein GF-120 bait and MAT blocks in an holistic manner.

Abstract ID 53

Regulatory Control at Irradiation Facilities

Elizabeth RENTERIA GERMANA

Nuclear Regulation Authority, Buenos Aires, Argentina, Email: *erenteria@arn.gob.ar*

With the creation of the *Fight Against Mediterranean Fruit Fly Committee* in the late 80's in Argentina, began the use of nuclear energy to control pests in different parts of the county. The technique used is the Sterile Insect Technique (SIT), using gamma irradiation to perform the sterilization, thereby interfering in the reproduction of the pest population in a way that does not affect the environment;.

Currently, Argentina has two mobile irradiators dedicated to this activity. The eradication of this pest in the provinces where this technique is used was crucial for the opening of new commercials activities for fruits and vegetables. Patagonia and parts of Mendoza Province have been proclaimed to be a Mediterranean fruit fly free due to successful application of this technique.

The global health emergency caused by the Zika, Dengue and Chikungunya virus, being *Aedes aegypti* the insect vector, encouraged efforts to find alternatives to control this pest. At present, research is ongoing to develop the SIT to control this vector in different parts of the world, including Argentina.

The present work is focused on a regulatory perspective of these irradiation facilities, describing their characteristics and risks. From the current regulatory framework, it presents the facility and a description of the personnel licensing system, the process of evaluation of the documentation, and the control system to be applied.

Abstract ID 71

Social Factors Influencing the Area-wide Management of Fruit Fly and the Use of Sterile Insect Technology

Aditi MANKAD[1], Barton LOECHEL[1] and Penelope MEASHAM[2]

[1]Commonwealth Scientific & Industrial Research Organisation (CSIRO), Canberra, Australia, Email: *aditi.mankad@csiro.au*
[2]Horticulture Innovation Australia (HIA)

Background: The area-wide management (AWM) of highly mobile insect pests, such as fruit flies, is a key biosecurity issue and requires an integrated understanding of relevant social and institutional processes that exist within that context. This series of qualitative studies examined stakeholder and community attitudes towards AWM to control Queensland fruit fly (*Bactrocera tryoni*) and the use of Sterile Insect Technology (SIT) as a tool used in conjunction with AWM.

Methodology: Fifteen focus groups (n = 78) and 35 one-on-one interviews were conducted across three dominant horticultural regions in Australia. Regions had varying levels of *B. tryoni* prevalence, ranging from zero to endemic. Research participants included growers, extension officers, industry, government representatives, and members of the general public. Participants were asked to consider potential barriers and facilitators to the widespread uptake of AWM integrating the SIT, and any relevant institutional issues.

Results: Combined data showed significant social barriers exist relative to the uptake of AWM and the SIT. Most notably, there were perceptions of low efficacy in successfully coordinating key social groups for the purposes of an area-wide approach, and a concern for the possibility of 'free-riders' within an area-wide system.

Lack of AWM efficacy was driven by fears that many people would have little or no motivation to change their behaviours, stemming from limited knowledge of the *B. tryoni* threat and its consequences. The most dominant barrier was a perception that AWM and the use of SIT would require significant economic and behavioural costs for participating individuals.

Participants also identified key facilitators that could assist in the uptake of AWM using the SIT, such as ways to increase social and community awareness. Growers and the broader community, in particular, indicated a need for more information about *B. tryoni* in general (e.g. its life cycle, overwintering, laying habits, consequences), to raise awareness of its significance as a horticultural pest. Growers and stakeholders cited the importance of trustworthy information sources and the persuasive influence that community champions and central packing sheds had on commercial growers.

All participant groups called for greater access to simplified scientific evidence demonstrating the effectiveness of innovations such as AWM and the SIT, which they believed would make the public more likely to engage in AWM and more accepting of SIT application. There was high general acceptance for the SIT, both on-farm and in towns, as long as the SIT was economically feasible at individual household level and the community was adequately consulted.

Conclusion: This research contributes to an understanding of key social barriers and facilitators for the successful implementation of an AWM programme targeting *B. tryoni* and integrating the use of the SIT as a key control measure.

Results will be used to inform implementation of best management practices (BMP) for the area wide control of *B. tryoni* in south-eastern Australia and the effective delivery of the SIT.

Abstract ID 72

Industry-driven Area-wide Management of Commercial Pests: Can it Work?

Heleen KRUGER

Plant Biosecurity Cooperative Research Centre (CRC), Australian National University, Acton, Sydney, Australia, Email: *heleen.kruger@anu.edu.au*

Background: Governments are increasingly challenged by limited budgets and associated staff cuts, whilst facing considerable biosecurity responsibilities. In Australia, this has contributed to an increased push for industry to take the reins of fruit fly area-wide management (AWM) programmes.

Methodology: The research presented is based on a PhD project that explored the key social and institutional considerations, constraints and opportunities for industry-driven fruit fly area-wide management in Australian horticulture industries. In the market access context, it focuses on achieving areas of low pest prevalence rather than pest free areas.

Findings are derived from three case studies where industry-driven AWM has been achieved or is being attempted. These involved 42 semi-structured interviews with key local stakeholders, three focus groups and a grower survey. Another 33 semi-structured interviews were carried-out with key informants working in the broader support system, including representatives of three levels of government, peak industry bodies, universities, researchers and consultants. This work draws on social theory about socio-ecological systems and agricultural innovation systems.

Results: Key findings include that the local social profile affects the ability of local industries to achieve AWM. If no formal measures exist to enforce compliance and cooperation, AWM is likely to be challenged by free-riding and people opting out if other pest risk contributors fail to cooperate. Key local social factors include the homogeneity or heterogeneity of growers; established trust and communication networks; existing social mechanisms that allow for monitoring compliance; as well as the ratio between the number of growers pursuing AWM and the number of risk contributors with limited incentive to manage fruit fly. Social and institutional factors influence the transaction costs of establishing and maintaining AWM. These are easily overlooked in conventional cost-benefit analyses of AWM.

Ways forward include the application of adaptive co-management thinking. This comprises groups connecting with various experts to draw on different knowledge systems (e.g. fruit fly behaviour and management, market access, community engagement) and to engage in on-going social learning, including ‘learning-by-doing’ and ‘leaning-by-interacting’. This is needed to develop and maintain an AWM programme that has strong adaptive capacity and is fit for the local context.

Complementary policy instruments and behaviour interventions fit for a specific context can overcome the limitations of voluntary approaches, without heavy on-going government investment. In short, the case study research shows that industry will need 'help to help themselves'.

More broadly, other challenges identified include a lack of local capacity, a disconnect between local AWM programmes and the national fruit fly governance system, and a lack of feasibility signals if AWM is to be applied in the context of systems approaches for market access. Investment in capacity-building programmes, knowledge brokers and innovation platforms can alleviate some of these challenges.

Conclusion: Achieving industry-driven AWM is a complex matter and local industries will need different forms of support to make such programmes feasible.

Abstract ID 74

A Predictive Psychosocial Model of Acceptance for the Area-wide Management of Fruit Fly and the Use of Sterile Insect Technology

Aditi MANKAD and Barton LOECHEL

[1]Commonwealth Scientific & Industrial Research Organisation (CSIRO), Canberra, Australia, Email: *aditi.mankad@csiro.au*

Background: Area-wide management (AWM) with the use of sterile insect technology is a modern approach to delivering insect pest control. The success of an AWM programme is dependent upon the coordinated efforts of key participants (e.g. growers, community members) within the designated area. However, public views regarding AWM may not necessarily reflect those views held by scientists or stakeholders; public considerations for acceptance are likely varied and multidimensional. Furthermore, stated acceptance for AWM among community members may not necessarily extend to public acceptance for the use of sterile insect technology (SIT), which is an innovation likely to elicit its own unique attitudes and beliefs.

This study examines drivers for the prediction of AWM and the SIT acceptance separately, and determines key factors important in innovation acceptance.

Methodology: A survey was conducted across three major horticultural regions across south-eastern Australia, comprising fruit growers and the general public (N = 1027). The 20-minute survey was administered via computer assisted telephone interviews; the general public group were recruited using a randomised search, and growers were recruited through combination of stratified and snowball sampling.

Results: The first psychosocial model tested examined public support for the use of AWM based on key innovation characteristics: relative advantage, compatibility, complexity, feasibility, observability and support. This model was found to explain 33% of acceptance for AWM, accounting for a moderate but significant amount of variance. The most influential predictor of acceptance was perceived complexity of AWM; that is, if the principles of AWM were easy to understand and apply, then participants were more likely to support the implementation of AWM. The second-most influential factor was observability, indicating a need for participants to see the results/outcomes of an AWM approach.

The second model examined social normative influences on acceptance of the SIT. This model explained 67% of the variance in the acceptance of SIT application and was a significant and powerful predictive model. Descriptive norms were found to be the strongest predictors of SIT acceptance, indicating that people were more likely to accept the SIT if they believed that important others (e.g. family, friends) would also support SIT implementation. Personal norms (i.e. feelings of moral obligation) were also found to be strong drivers of acceptance for the SIT.

Conclusion: Acceptance for AWM and the SIT was significantly predicted from key psychosocial variables, highlighting key social drivers of innovation acceptance. The results highlighted the most influential factors for social acceptance of AWM and the use of the SIT, which has implications for the design of future communication and community engagement strategies. Results also suggested that all participants within the targeted communities were invested in decisions around AWM and the use of the SIT, not just the growers themselves.

Future research could evaluate how AWM and SIT initiatives could be framed and communicated to the general public so as to maximise this sense of inclusivity and shared responsibility.

Abstract ID 168

Pesticide Management in Turkey

Yunus BAYRAM and Dilek DERVİŞOĞLU İÇEL

Ministry of Food Agriculture and Livestock, General Directorate of Food and Control, Ankara, Turkey, Email: *yunus.bayram@tarim.gov.tr*

Plant Protection Products (PPPs) are authorized by the Ministry of Food, Agriculture and Livestock, in order to be used against pest organisms that are available in growing areas or storage places. In Turkey there are 363 active substances and nearly 4643 PPPs, but only 1,500 to 1,600 PPPs are available on the market. There are 464 companies engaged in plant protection products, of which 174 actively carry out this work. There are 65 PPPs manufacturer in our country.

In the event that there is any controversy about any production, such as change or difference in their registration; quality, packaging, labels, prospectus, information, promotional posters and leaflets, physical and chemical analysis or sales in violation of regulations, labels that passes the limit of tolerance, then the corresponding company would be punished according to their categories as described in numbered 5996 law and related regulations.

In 2015 nearly 50.000 to 55.000 tons of raw materials have been imported. In addition, about 10.000 to 15.000 tons of products such as pheromone traps, biotechnical and biological control productions, plant growth regulators, microorganisms, production that contain cooper and sulphur and some pesticides for exporting purpose have been imported. Inspections and procedures of authorization, manufacturing, importing, marketing and controlling of PPP in Turkey are generally made according to EU regulations.

The "*Plant Protection Products Data Base Programme*" has been established since 2014 by the Ministry of Food Agriculture and Livestock and this programme has all necessary information for nearly 4,643 authorized PPP. In addition, this programme has an English version with main titles, and its mobile application has been operated since April 2016. All import processes have been made electronically. Moreover, company representatives always could inquiry and follow electronically their import applications. The "*Plant Protection Products Data Matrix Tracking Programme*" has been established and by this programme all PPPs will be tracked with the same code system from produced factory to wholesaler, dealers and farmers.

In order to assure the safe food supply from farm to fork and to address the residue problem in fresh fruits and vegetables, "*Pre-Harvest Pesticide Control*" is performed by the Ministry in production areas such as orchards, vineyards, cropland and greenhouses since 2012, before the products are supplied to the market. The penalty of food destruction or delaying harvest is imposed to the products in which defective PPPs use is determined as a result of the control

and administrative fines are imposed to the product owners. In 2015, 9,435 samples were analysed for pre-harvest controls.

Post-harvest controls are carried out in packaging facilities, wholesale and retail outlets. Official controls for pesticide residues in fresh fruits and vegetables are carried out throughout the country as part of the annual food control plan, which is being drafted and includes the recommendations on the coordinated monitoring programme prepared by the EU Commission on an annual basis. This information and the products with high production and consumption quantities, the fresh fruits and vegetables with import and export volumes, are given with the statistics on the web page of the Food Safety Information System (GGBS).

Key Words: Pesticides, PPPs Data Matrix Tracking System, pre-harvest and post-harvest control, Turkey

Abstract ID 282

Non-host Status as a Component of Area-wide Pest Management for Banana and Papaya to Prevent *Bactrocera dorsalis* Infestation and Facilitate Market Access

Domingos CUGALA[1] and Sunday EKESI[2]

[1]Faculty of Agronomy and Forest Engineering, EMU, Maputo, Mozambique, Email: *dcugala@gmail.com*
[2]The International Centre of Insect Physiology and Ecology, Nairobi, Kenya

Background: Fruit flies (Diptera: Tephritidae) are globally recognized as the most destructive insect pests of fruits and vegetables. The invasive fruit fly *Bactrocera dorsalis* is one of the most destructive in the genus *Bactrocera*. The invasion and spread of *B. dorsalis* throughout Africa in recent years has caused serious economic losses as a result of both direct fruit damage as well as the loss of lucrative regional and international export markets due to quarantine restrictions imposed by importing countries.

Demonstrating non-host status of fruit can be an important element of pest risk analysis to gain commodity market access in the presence of fruit flies including *B. dorsalis*.

Methodology: Field trials under semi-natural conditions were carried out to assess the infestation of *B. dorsalis* on 'Dwarf Cavendish' bananas and 5 cultivars of papaya of different ripening stages in Kenya and Mozambique at different densities of *B. dorsalis* females.

Results: On banana, fruit fly infestation was recorded from the "100% yellow" ripening stage. *B. dorsalis* was observed attacking fruits when papaya fruits were at 75% yellow (fruits at three-quarter ripe) and 100% yellow (fully ripe fruits) with infestations of 0.19-0.51 and 0.24-1.24 *B. dorsalis*/kg fruit, respectively, in all the locations. In field cage experiments conducted in Kenya and Mozambique, although oviposition wounds (101-587) were observed on green bananas after artificial infestation with 100-1000 gravid *B. dorsalis* females, no flies were recovered from fruits at the green stage of harvest maturity.

On the papaya cultivars 'Papino', 'Neo Essence', 'Sunrise Solo', 'Tainung No. 1', and 'Tainung No. 2' in Manica province, Mozambique, *B. dorsalis* did not infest fruits at ripening stages of 0%, 25% and 50% yellow at the densities of 50 and 100 flies per cage. However, at 75% yellow, up to 13.1 pupae/kg of fruits were recorded at a density of 150 flies per cage in Tainung No. 1, and infestation ranged from 4.5-136 pupae/kg fruits at 100% yellow across all the cultivars and infestation densities.

Conclusion: Based on the results mature green bananas of the 'Dwarf Cavendish' cultivar harvested at less than 40% yellow should be considered as non-host for *B. dorsalis*. However, bunches of banana with precociously ripened fingers and with damages should be culled

before export as such situations can permit infestation by *B. dorsalis* and pose risk of inadvertent movement of the insect during export.

The results also suggest that harvesting papaya fruits at less than 40% yellow stage should avoid the need for quarantine treatment because *B. dorsalis* did not infest papaya fruits at those stages and, therefore, should permit access to quarantined markets.

Key words: Bactrocera dorsalis, *'Dwarf Cavendish' banana, papaya, non-host, ripening stages*

Abstract ID 291

The Importance of the Pervermac II Project in the Sustainable Use of Pesticides and its Impact on Human Health in the Macaronesian Area: The Azores Case

David HORTA-LOPES[1], Andreia AGUIAR[2], Ana DIAS[3], Maria BETTENCOURT[3], Ricardo DUTRA[3], Reinaldo PIMENTEL[1]

[1]Azorean Biodiversity Group (GBA, CITA-A) and Platform for Enhancing Ecological Research & Sustainability (PEERS), Universidade dos Açores, Faculdade de Ciências Agrárias e Ambiente, Terceira, Azores, Portugal, Email: *david.jh.lopes@uac.pt*
[2]Unidade de Saúde da Ilha Terceira (USIT), Centro de Saúde de Angra do Heroísmo (CSAH), Terceira, Azores, Portugal
[3]Escola Superior de Saúde, Departamento de Enfermagem, Saúde Mental e Gerontologia, Terceira, Azores, Portugal

The overall objective of the PERVERMAC II project is to promote food security and responsible agriculture in the archipelagos of the Macaronesian region (Azores, Madeira, Canaries and Cape Verde), with the aim of ensuring the safety and health of farmers and consumers, minimizing the risk of health and environmental contamination.

In this sense, the level of exposure of consumers to the application of pesticides will be determined through the quantification of the levels of residues in the agricultural products from the main crops. Those products collected using programmed sampling actions for fruits, vegetables, tubers, wine and cereals from our food diet will contribute through their analysis to the food security and the safeguarding of the population. At the same time, by conducting food surveys and determining the amount consumed, we will obtain the real proportion of these products in our diet.

If residues found are above the tolerable levels, on the products analysed, others should be used through the promoting good food practices, and sensitizing the populations of the regions and countries involved in their adoption with a special focus on the school population, widening their food components knowledge and their awareness of the implementation of school gardens with organic production.

The developing of actions at the level of the youngest, in schools and in the population in general through practical workshops demonstrating healthy eating habits, combined with the development of a manual of good food practices, are important means of dissemination and adoption of healthier and safer food diets.

Regarding farmers and technicians, actions for the transfer of knowledge and technology are programmed through demonstration actions and specific training. Also the elaboration of a manual of good and sustainable agricultural practices will ensure the adoption and implementation of integrated production / protection measures, promoting the sustainable use

and the current environmental impact of pesticides by implementing alternative means of combating the major and most frequent phytosanitary problems of these crops.

This work has been funded by resources from the European Fund for Regional Development (FEDER), articulated through the Territorial Cooperation MAC 2014-2020.

Poster Presentations Session 5: Climate Change, Global Trade and Invasive Species

Abstract ID 39

Use of Radiation in Phytosanitary Treatment and Control of Quarantine Pests

Inamullah KHAN, Zahid MUHAMMAD and Alam ALAMZEB

Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan / Pakistan Atomic Energy Commission (PAEC), Islamabad, Pakistan, Email: *inamullah17@gmail.com*

Background: Quarantine pests are of potential economic importance to the endangered area where they are not yet present, or present but not yet widely distributed and being officially controlled. These pests inflict both direct losses to the fruits and vegetables and indirectly pose problems in the export of fresh fruits and vegetables to the pest-free countries as the eggs and juvenile stages can be carried with the export commodity to the importing countries.

Citrus and mangoes are the top exporting fruits of Pakistan due to their high acclaimed taste and quality attributes. The citrus psylla, *Diaphorina citri*, and scale insects (mango scale *Aspidiotus destructor*, and citrus red scale *Aonidiella aurantii*) are the key pests of these fruits in Pakistan, but regulated in many parts of the world. Pakistan consequently loses export of these fruits. Currently very few researches in Pakistan have focused on this problem.

Methodology: Studies on the use of gamma irradiation as a phytosanitary treatment in citrus and mangoes for the mortality and growth inhibition of *D. citri*, *A. destructor*, and *A. aurantii* were initiated under the Coordinated Research Project (CRP-16894) of IAEA. The effects of gamma irradiation from a Cobalt-60 source were examined to disinfest fruits. Dose response tests were conducted with eggs, 1^{st}, 2^{nd} and 3^{rd} stage nymphs, and adult female scales without eggs and with eggs using a series of radiation doses between 100 and 300 Gy.

Results: In general, irradiation affected all life stages of *D. citri*, *A. destructor*, and *A. aurantii*. The pattern of tolerance to irradiation in all these insects was eggs <1st instar<2nd instar<3rd instar and adults. The adult female in *A. aurantii* with and without eggs irradiated at 220 Gy did not produce F_1 generation with eggs. Similarly, a dose of 217 Gy was determined to completely stop *A. destructor* development to subsequent stages.

Conclusion: Results from validation tests indicated a minimum of 200 Gy dose for 99.99% inhibition of *A. aurantii* and *A. destructor*, and therefore, recommended to provide quarantine security to citrus and mango fruits.

Key Words: Quarantine, phytosanitary, export, scale insects, citrus psyllids, radiation, control

Abstract ID 78

The Establishment of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in Botswana

Casper NYAMUKONDIWA and Rebaone MOTSWAGOLE

Botswana International University of Science and Technology, Palapye, Botswana, Email: *nyamukondiwac@biust.ac.bw*

Background: Fruit and vegetable production is one of the fastest growing agricultural sectors in Botswana, providing incomes, employment and general livelihoods. However Tephritid fruit flies, e.g. *Bactrocera dorsalis*, hinder economic production of these fruit trees and thus economic transformation of livelihoods through agriculture.

Since its African discovery in 2003, and first introduction in Botswana (2010), *B. dorsalis* still represents a significant biosecurity threat in agro-based communities. This is because, since its introduction, no work has documented its further spread south of Chobe district, the area of first report.

Methodology: Through a two year surveillance study (2015-17), we investigated the nationwide distribution of the invasive *B. dorsalis* following first detection. Methyl-eugenol lures were used as attractants in conjunction with yellow bucket traps. The buckets were placed strategically on fruit plants with a minimum required trap density of 1 trap per km^2, and repeated in all districts. An insecticide block Dichlorvos (DDVP) was added in each bucket to kill the trapped flies and prevent them from escaping. The buckets were placed on trees 1.5 m above the ground and protected from the sunlight and strong winds, and coated with ant barrier for further protection. Traps were serviced fortnightly by changing the attractant, and then be opened to check for any specimen caught.

Commercial and wild fruit within the sampling sites were also collected, incubated and any emerging adult flies identified. All specimens were identified using morphology in the laboratory.

Results: Preliminary results indicated presence of adult *B. dorsalis* all year round in Chobe (district of first detection). In addition to Chobe, consistent populations were observed in Ngamiland, Central, North east, South East, Southern, Kweneng and Klateng districts, indicating the spread and establishment of the invasive insect further south.

Conclusion: These results are important for phytosanitary regulations, invasion biology and may help in integrated pest management. Future work should look into the optimization of monitoring and control techniques for efficacious *B. dorsalis* management in Botswana. Furthermore, release of parasitoids may also assist in controlling this pest, especially in the 'wild' and non-commercial agricultural environments.

Abstract ID 82

Development of the Sterile Insect Technique to Manage Confined Populations of *Drosophila suzukii*

Fabiana SASSU and Carlos CÁCERES

Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria, Email: *fabiana.sassu@gmail.com*

The Spotted Wing Drosophila fly (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), is a destructive insect pest of fruit crops. Although most of its closely related sibling species are not fruit pests, the serrated ovipositor of the SWD permits ovipositing in undamaged fresh fruits. The invasion of SWD into Europe and America has had a severe impact on the farming economy caused by its polyphagous habit, remarkable reproductive potential and fast development. As is the case with most insect pests of food in the world, the SWD is mainly controlled using insecticides, which is a serious public concern. There is an increasing demand for the replacement of the intensive use of chemicals by environmentally-friendly sustainable methods and their application within Area-wide Integrated Pest Management (AW-IPM) approaches.

Because SWD represents a serious threat and there is a lack of appropriate control methods, a project entitled “SUZUKILL” was initiated with a focus on biocontrol of SWD using innovative biotechnological approaches, such as the sterile insect technique (SIT) and the incompatible insect technique (IIT) associated with cold tolerance management. One of the general goals of the SUZUKILL project is to develop alternative and innovative protocols that include the release of sterile insects to biologically control SWD, initially in greenhouses. The SIT is a species-specific, non-polluting, and resistance-free genetic approach. Generally, males are mass-reared, sterilized by exposure to ionizing radiation and released in target areas where they compete with fertile wild males for wild females. Mating of sterile males with wild females resulted in no offspring, which eventually will reduce the pest population and the infestation.

The SIT can only be applied efficiently against SWD when the insect can be adapted to laboratory conditions and protocols are available for the cost-effective mass-rearing and quality control. However the application of the SIT requires also studies on irradiation to determine the optimal reproductive sterilization dose that will not compromise the quality and performance of the released sterile insects in the field. Due to the high and fast reproductive potential of SWD, the SIT will be initially considered to manage this pest in confined environments, such as greenhouses. The success of this research to develop the SIT to manage confined populations will contribute to the development of innovative, practical and biological solutions to better manage the threat of *Drosophila suzukii*.

Abstract ID 87

Survey on Exotic Fruit Fly Species in Romania

Constantina CHIRECEANU[1], Andrei TEODORU[1], Andrei CHIRILOAIE[1] and Cornel TEODORESCU[2]

[1]Research and Development Institute for Plant Protection Bucharest, Romania, Email: *cchireceanu@yahoo.com*
[2]Nuclear Agency & Radioactive Waste, Bucharest, Romania

Background: In Romania, exotic fruit flies represent a real threat to fruit and vegetable production, as they do in many other countries from the Balkans and Eastern Mediterranean regions. The Mediterranean fruit fly, *Ceratitis capitata*, a highly invasive species, inflicts the greatest risk on fruit production in this area. As a result of an increasing trend of global warming, *C. capitata* could extend its optimum development area towards the Northern part of the Balkans and East Mediterranean zones, in which Romania is situated as well.

For the early detection of the Mediterranean fruit fly and quarantine fruit flies in Romania, we have run a fruit fly survey and monitoring programme within the framework of three TC regional projects RER5018/RER5020/RER5021 under the coordination of IAEA Vienna.

Methodology: The survey activity was conducted during the period 2013-2016 following the protocols described in the trapping guidelines for fruit fly provided in the FAO/IAEA. Three types of traps were used: Tephri traps with Biolure (trimethylamine, ammonium acetate and putrescine) plus toxicant DDVP, Jackson traps with methyl-eugenol plug, and Jackson traps with trimedlure plug. The traps were placed in fruit trees in important fruit growing areas and backyards of houses and institutions, botanical gardens and experimental fields in different parts of Romania.

Results: Trapping data of the 4 years of survey indicated no quarantine fruit flies (such as *Bactrocera* spp.) detected in Romania. The Mediterranean fruit fly *C. capitata* was detected in a small number (1-6 specimens/site) in each year of survey, in Tephri traps with Biolure and Jackson traps with trimedlure, in all type of emplacement (fruit orchards, houses backyard, botanical gardens and experimental fields) from the end of August to the end of October. No specimen was found in traps on sweet and sour cherry trees. Only in the traps placed on the Chinese date trees in an experimental field in Bucharest city an observable amount of adults of *C. capitata* was recorded. The adult population trapped was variable from one year to the other. The total annual captures ranged between 2 and 202 flies.

In September 2013, the first adults of *Drosophila suzukii* captured in Romania were found in a Tephri trap with Biolure placed on wild blackberry in Bucharest city (Southern Romania). Also two species of *Carpomya*, *C. schineri* and *C. vesuviana*, were caught in Tephri traps with Biolure.

Conclusion: During the 4-year survey period (2013-2016), no quarantine fruit flies were detected in Romania. *C. capitata* was captured in traps placed on fruit trees (apricot, peach, plum, and apple) in West, South and South-Eastern Romania, from the end of August to the end of October, except for sweet and sour cherry traps. Notable *C. capitata* captures took place in traps on Chinese date trees in Southern Romania. The presence of *D. suzukii* was given evidence in 2013 in Southern Romania. *C. vezuviana* and *C. schineri* species were also captured.

Abstract ID 93

An Updated Multi-entry Identification Key to African Fruit Infesting Dacines (Diptera, Tephritidae: Dacini)

Massimiliano VIRGILIO, Franck THEETEN, Son DU, Benoît HARDY, André DE MÛELENAERE and Marc DE MEYER

Royal Museum for Central Africa, Tervuren, Belgium, Email: *massimiliano.virgilio@africamuseum.be*

The morphological identification of African Dacines (including representatives of widely distributed pests) relies on classical single-access (dichotomous) keys published in specialised taxonomical journals. These keys are not easy to use by the non-specialist and the identification inevitably fails whenever the user is not able to score any of the dichotomous character states (because of absence of the character or unclear description of the character state).

For this reason we developed a multi-media and multi-entry identification key for African fruit-infesting Dacines that could facilitate the morphological identification by non-specialists. The multi-entry key, available on a *Lucid3* platform, is a self-contained and easily accessible tool with a total of 396 African tephritid species, including exotic invasive and all taxa of economic significance.

The key includes a "pre-key" for genus identification and individual keys to representatives of nine fruit fly genera in Africa (*Bactrocera*, *Capparimyia*, *Carpophthoromyia*, *Ceratitis*, *Dacus*, *Neoceratitis*, *Perilampsis*, *Trirhithrum*, *Zeugodacus*). Separate character sets are considered for each genus (range 11-95 characters, 22-280 character states) and images and drawings are provided for characters, character states and species. A number of filters allow selecting species by region or excluding characters of more difficult scoring.

The formal species description is provided according to the published scientific literature and information regarding taxonomic status, collection specimens, geographic distribution within Africa and DNA barcoding is available through hyperlinks to the *Encyclopedia of Life*, to the *Belgian Biodiversity Platform* (as Belgian portal to GBIF) and to the *Barcoding of Life Database* (BOLD).

The key is accessible online or downloadable for offline use through the website of the Royal Museum for Central Africa (https://fruitflykeys.africamuseum.be/). Its potential as an android app is currently being considered.

Abstract ID 96

Large Phytosanitary Irradiation (PI) Dedicated E-beam Facility Set Up at Ports

Huaili QIN, Guang YANG and Cancan LI

Nuctech Company Limited, Beijing, China, Email: *qinhuaili@nuctech.com*

Imported items such as fruits and woods carrying exotic pests will cause damage to the importing countries' farming. Thus, the prevention and control of exotic organisms relate directly to the ecological security of the importing countries. Irradiation has been used for phytosanitary purposes for decades. The International Consultative Group on Food Irradiation (ICGFI) supported by FAO, IAEA and WTO found that quarantine radiation treatment is an effective and widely applicable quarantine approach. E-beam irradiation has been proved as effective and efficient way for area-wide management of insect pests.

An effective quarantine at the China-ASEAN border is necessary and imperative, as the amount of ASEAN fruit imported to China has been growing rapidly. As China's largest import and export port for ASEAN fruits, the annual amount of fruits entering through Pingxiang, Guangxi has been increasing from 0.5 to 0.7 million tons (2011-2015).

Under this situation, the largest dedicated phytosanitary irradiation (PI) E-beam facility in the world, the *China-ASEAN Pingxiang Fruit Irradiation Quarantine Processing Center* (ca. 2000 m^2), has been established on the border of China and Viet Nam. Two 10MeV/7.5kW accelerators are applied, with one on the top and another on the bottom of the conveyor, allowing for double-side irradiation of fruits. The system has 7 transporting lanes, allowing a maximum of 14 trucks working simultaneously on the loading and unloading area.

Precise delivery is guaranteed with high-precision bar code identification device on each lane so that fruits can be delivered sequentially to the irradiation zone in the best speed. The system could satisfy demands for a wide range of surface doses (200-18 kGy per single irradiation) and effectively guarantee process quality and easy production management. The processing capacity can be ca.100,000 tons/year.

This center will help with the prevention and control of exotic organisms and be beneficial to the ecological security of the importing countries, promote the fruits trade between China and ASEAN, and set a good example for the world's border trade.

Abstract ID 98

Dealing with Spotted Wing Drosophila: New Insights on Suzukii Trap® Bait Performance World-wide

Anna BOTTA[1], Cándido MARÍN[1], Nuria SIERRAS[1] and Richard COWLES[2]

[1]R&D Bioibérica, Barcelona, Spain, Email: *abotta@bioiberica.com*
[2]The Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA

Since the recent establishment of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in berry crops all over the world, different attempts are being developed to control their invasive populations. Various strategies such as insecticide treatments, mass-trapping or attract and kill practices are being considered in integrated production.

Monitoring of populations, early detection and damage threshold establishment with an effective attractant and trap has become essential for managing this invasive pest. A new Suzukii Trap® bait formula has been developed to specifically capture this new pest, with early detection, high efficacy and selectivity (avoiding capture of non-target insects) and with a relatively high percentage of females captured.

Screening bioassays and field trapping experiments with Suzukii Trap® bait conducted last year in variety of conditions comparing with other attractant and systems are being presented. Also, the ease and efficacy of implementing different control systems for spotted wing drosophila in integrated pest management is discussed.

Experiences suggest the need to further improve and to test complementary management tools to enhance biotech's role in reducing crop damage caused by this fruit fly in sensitive crops.

Abstract ID 100

Monitoring of the Spotted-Wing Drosophila (*Drosophila suzukii* Matsumura) in Romania

Andrei TEODORU, Constantina CHIRECEANU and Andrei CHIRILOAIE

Research and Development Institute for Plant Protection, Bucharest, Romania, Email: *andrei_theodor44@yahoo.com*

Background: The Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae) is an invasive vinegar fly native to Asia, which was introduced to Europe in 2008. This species damages the thin-skinned fruits, being considered as one of the most serious pest for fruit species such as cherries, strawberries, raspberries, black and blueberries, stone fruits, as well as grapes.

First adults of *D. suzukii* were found in Romania in September 2013 in a Tephri Trap with Biolure (3 components: trimethylamine, ammonium acetate, putrescine) placed on wild blackberry bushes in an area in northern part of Bucharest city (Southern Romania).

Methodology: The following years after its detection, field monitoring activities have been initiated in order to collect important data on its host fruit species and potential for spreading, as well as elements of its biology in our country. During the period 2014-2016, seven sweet and two sour cherry orchards, two peach and two plum orchards and two vine plots were surveyed for the presence of *D. suzukii* adults, using traps consisting of 0.5 l plastic bottles, baited with apple vinegar and red wine mix (1:1). Traps were also placed on wild blackberry bushes. Fruit samples were collected to determine larval presence.

Results: D. suzukii adults were captured in the traps placed in one plum orchard and in one grapevine plot and on wild blackberry bushes in the autumn season. No adults were collected in the traps on the sweet and sour cherry orchards during the spring and summer seasons. Adults of *D. suzukii* have been emerged under laboratory conditions only from the fruit samples of wild species, namely wild blackberry (*Rubus caesius*), European dwarf elder (*Sambucus ebulus*) and pokeweed (*Phytolacca americana*).

In 2014, we collected in the traps placed on wild blackberry, one hundred adults of *Zaprionus tuberculatus* another invasive fruit fly species belonging to the Drosophilidae family.

Conclusion: Adults of *D. suzukii* were captured in traps placed in plum and grapevine lots and wild blackberry bushes. No adults were trapped in the traps in sweet and sour cherry orchards. No infested fruits belonging to cultivated species were found. The only infested fruits found were from the spontaneous flora: wild blackberry, European dwarf elder and pokeweed.

Abstract ID 102

Establishing a Generic Radiation Dose as Post-harvest Phytosanitary Treatment against Bruchid Species (Coleoptera: Chrysomelidae) Infesting Legumes

Ranjana SETH[1], Rakesh Kumar SETH[1], Basavaraj PATIL[2], Rachappa HAVERI[2], Shivanand HANCHINAL[2], Mahtab ZARIN[1] and Zubeda KHAN[1]

[1]Applied Entomology and Radiation Biology Unit, Department of Zoology, University of Delhi, Kalkaji, New Delhi, India, Email: *seth.ranjana.27@gmail.com*
[2]University of Agricultural Sciences, Raichur, Karnataka, India

Background: The bruchids are globally distributed with a wide host range and can cause immense quantitative and qualitative losses in grain legumes. The quarantine risk in trade and exchange of germplasm is mainly due to the hidden nature of infestation caused by these pests. Among bruchids, *Callosobruchus* species are frequently detected in trade consignments in India. It is very challenging to control the larval stages (internal feeders) of bruchid species with insecticides. Hence, an interest in the use of irradiation as an eco-compatible phytosanitary treatment is growing worldwide.

In the present study, the bioefficacy of radiation was assessed against three bruchid species with an aim to determine generic dose as post-harvest phytosanitary treatment against the bruchid species having quarantine status.

Methodology: Radiobiological studies were conducted on three bruchid species- *Callosobruchus maculatus, C. chinensis* and *C. analis*, in terms of (a) assessing age correlated radio-sensitivity of different ontogenic stages of each species, (b) ascertaining the effect of sub-lethal gamma doses on various bio-characteristics of each bruchid species, (c) determining phytosanitary irradiation (PI) gamma dose range by identifying the most radio-resistant pre-imaginal stage (with respect to metamorphic inhibition and prevention of adult formation) and highly radio-resistant imaginal stage (with respect to inducing sterility). Large scale testing of radiation efficacy was conducted using species specific PI dose, and generic dose for disinfestation of the pest species complex.

Results: Pharate adult as the most radio-resistant pre-imaginal stage required a higher dose to cause metamorphic inhibition than the dose required to induce sterility in the highly radio-resistant imaginal stage (4-5 day old male adult). The gamma dose causing metamorphic inhibition in pharate adult (late pupa) was 141 Gy, 181 Gy, and 134 Gy in *C. maculatus, C. chinensis*, and *C. analis*, respectively.

Further, the dose required to induce sterility in 4-5 day old adult males (crossed with unirradiated females) was 117 Gy, 123 Gy, and 102 Gy in *C. maculatus, C. chinensis*, and *C. analis*, respectively.

However, in order to completely check oviposition (that is otherwise permissible as per the international phytosanitarty standards), the radiation dose needed was 339 Gy, 352 Gy, and 205 Gy in *C. maculatus*, *C. chinensis*, and *C. analis*, respectively.

The PI dose ranged between 141 to 181 Gy for these bruchid species, and large-scale testing of PI for each bruchid species was conducted, followed by evaluation of 200 Gy as a generic dose for the management of *Callosobruchus* species complex.

Conclusion: Radio-responses among different ontogenic stages of the three bruchid species indicated their order of radio-resistance as *C. chinensis* > *C. maculatus* > *C. analis*. 200 Gy can be considered as a generic dose keeping in view of the relative response of the most radio-resistant pre-imaginal stage and the most radio-resistant imaginal stage. 200 Gy could effectively prevent adult formation in the parent and F_1 generation and induce sterility in these bruchid species. Large scale tests also validated the use of 200 Gy as an effective generic dose.

Abstract ID 111

Application of Ionizing Radiation for Abatement with Insect Pests of Grain and Grain Products during Storage

Nadezhda LOY and Natalia SANZHAROVA

Russian Institute of Radiology and Agroecology, Obninsk, Russian Federation, Email: *loy.nad@yandex.ru*

Under laboratory conditions, we investigated the influence of a wide range of doses of γ-, electron and bremsstrahlung radiation on the survival of *Tribolium confusum* Duv.

The results showed that gamma-irradiation of this small tenebrionid beetle (larval stage and adult beetles), inhabiting grain and grain products, in a range of doses of 700-1,000 Gy, caused pest mortality of 100% over 15 days after irradiation with a dose rate of 500 Gy/h and over 30 days with a dose rate of 120 Gy/h.

The use of the bremsstrahlung and electron radiation for treatment of barley infested with the tenebrionid beetle, caused mortality of 100% of insects over 15 days after treatment at any irradiation mode, excepting the mode with irradiation at a dose of 250 Gy in case of bremsstrahlung and at a dose of 1,000 Gy in case of electron irradiation; the irradiation effectiveness in these two cases was 90%. At all doses and dose-rates and both radiation types the mortality of 100% of tenebrionid beetle was detected over 30 days after treatment.

Nutrient content in barley grain changed variously at gamma irradiation: content of ash and solids decreased at a dose rate of 120 Gy/h (absorbed dose 800 Gy) and, on the contrary, at a dose rate of 500 Gy/h there was observed a statistically significant increase in the ash content on 3.6-6.5% at all investigated doses; cellulose content increased 19.8% in case of absorbed dose of 800 Gy.

Gamma-irradiation of semolina in the dose range 700-1,000 Gy (dose rate - 500 Gy/h) caused statistically significant reduction of ash content on 17.3% at a absorbed dose of 900 Gy and proteins on 8.1-7.7% at absorbed doses of 800 and 900 Gy, and the increase in fat content on 11% at the dose of 900 Gy.

The application of bremsstrahlung and electron radiation in the investigated dose range basically had no significant effect on the quality of barley grain of Nur variety compared with reference values.

Abstract ID 150

Managing Cold Tolerance and Quality of Mass-produced *Drosophila suzukii* Flies to Facilitate the Application of Biocontrol through Sterile and Incompatible Insect Techniques

Katerina NIKOLOULI[1], Fabiana SASSÚ[1], Thomas ENRIQUEZ[2], Patricia GIBERT[3], Fabrice VAVRE[3], Laurence MOUTON[3], David RENAULT[2], Christian STAUFFER[4], Carlos CÁCERES[1], Kostas BOURTZIS[1], Rui CARDOSO PEREIRA[1] and Herve COLINET[2]

[1]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria, Email: *k.nikolouli@iaea.org*
[2]UMR ECOBIO, Centre National de la Recherche Scientifique, Université de Rennes, France
[3]Université de Lyon, France
[4]BOKU-University of Natural Resources and Life Sciences, Vienna, Austria

Drosophila suzukii, a vinegar fly originated from South East Asia, has recently invaded western countries and it has been recognized as an important threat for a wide variety of commercial soft fruits both in open field and greenhouse production systems. The shape and length of the females' ovipositor allows *D. suzukii* to infest a wide range of ripening fruits, leading to substantial yield and revenue losses in the affected regions. The species demonstrated a rapid and widespread expansion in North and South America and in Europe, and it has escalated into a major invasive insect pest. The development of an efficient environmentally-friendly control approach to fight against *D. suzukii* is necessary. Insecticide application approaches were initially used to control this pest in the invaded areas. Nevertheless chemical control has proved to be an inefficient approach for *D. suzukii* exhibiting several drawbacks as a pest management tactic.

An encouraging way out is to employ species-specific and environmentally-friendly control methods, like the Sterile Insect Technique (SIT). The SIT has been successfully applied for almost 60 years in controlling various insect pest and disease vector species worldwide.

The SUZUKILL project (funded by ANR and FWF, French-Austrian Joint Projects) emerged following the requirement to develop an alternative avenue for controlling *D. suzukii*, initially in greenhouses, through the release of sterile insects produced for SIT application. As a contingency plan, we suggest to exploit *Wolbachia*, a bacterium able to manipulate its host reproduction, to develop the Incompatible Insect Technique (IIT)., which is based on cytoplasmic incompatibility. The SUZUKILL project aims to develop protocols for mass-rearing, quality control and sterilization of *D. suzukii* employing SIT and/or IIT.

The ability to preserve insects without loss of performance and to mobilize them quickly upon demand is essential for SIT and IIT and this can be performed under specific cold treatments. Mass-reared insects are also exposed to cold for immobilization and handling

during the rearing process and long-distance shipping is also performed under low temperature. Temperature in the release site may be stressful and this necessitates application of thermal conditioning protocols before release to prevent thermal stress-induced mortality. In consequence, a successful application of SIT and IIT requires basic knowledge on *D. suzukii* thermal biology in order to develop protocols to manipulate thermal tolerance.

In addition, under laboratory domestication and continuous mass-rearing, laboratory strains can lose their ability to survive and compete under natural conditions. Some of these changes have genetic basis as they result from inadvertent selection or bottlenecking in laboratory population. Assessing and controlling genetic changes as well as changes in the associated microbiota during laboratory domestication and continuous mass-rearing are thus a critical step of quality control.

The general goals of the SUZUKILL project are to: (i) develop alternative and innovative protocols to biologically control *D. suzukii* through SIT and/or IIT, (ii) manage thermal tolerance of insects to enable and facilitate application of SIT and IIT, and (iii) assess and control the genetic changes that occur under laboratory domestication.

Abstract ID 155

Using Hydrogen Stable Isotope for Tracing of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in China

Zihua ZHAO

China Agricultural University, Beijing Shi, China, Email: *zhzhao@cau.edu.cn*

Background: The Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), is a generalist feeder, known to successfully feed and breed on a variety of fruits and vegetables in tropical and subtropical zone of Asia. Recently, *B. dorsalis* specimens have been collected in the north of China (Beijing). However, the origin was not clear until now.

Methodology: In the present research, traps containing the attractant methyl-eugenol were used to collect *B. dorsalis* samples of first appearance in June by using random 5-point method in Fangshan district (Beijing, China). At the same time, *B. dorsalis* flies were also collected from populations in Haikou, Guangzhou, Fuzhou, Wuhan, Yixing by using the same attractant with the same sampling methods, which represent different geographical regions having a gradient of δ^2H stable isotope values.

The relationship between water and *B. dorsalis* δ^2H stable isotope values was established, which could be used to determine the population origin of *B. dorsalis* in Beijing, China.

Results: Results showed that the δ^2H stable isotope of *B. dorsalis* flies found in Beijing was not consistent to the water in Beijing, while it was consistent to that of Fuzhou (Fujian Province) in contrast. Therefore, we concluded that *B. dorsalis* samples collected in Beijing represent the first appearance of the pest, which until now has not become established locally, with the specimens originating from some provinces of South China.

Conclusion: The fruit and vegetable trade may be an important avenue causing northward extension of the *B. dorsalis* populations. Additionally, our results also proved that the δ^2H stable isotope technology is a promising technology to trace populations of invasive alien organisms.

Abstract ID 172

Radiation Dose Responses of *Drosophila suzukii* (Matsumura) for Use in the Sterile Insect Technique (SIT)

Annabelle FIRLEJ[1], Geneviève LANOUETTE[2], Marc VREYSEN[3], Carlos CACERES-BARRIOS[3], François FOURNIER[4], Véronique MARTEL[5] and Jacques BRODEUR[6]

[1]Institut de Recherche et de Développement en Agroenvironnement (IRDA), Québec, Canada
[2]Université de Montréal, Canada
[3]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria
[4]Collège Montmorency, Laval, Québec, Canada
[5]Ressources Naturelles, Canada
[6]IRBV

Background: The spotted wing drosophila, *Drosophila suzukii* (Matsumura), has recently invaded Europe and North America. This pest has a devastating effect on small fruit crops as it attacks a large variety of ripening fruits such as cherries, raspberries, strawberries, blueberries. Control of *D. suzukii* populations has essentially been based on applications of broad-spectrum insecticides.

Methodology: In an effort to assess the potential of using the sterile insect technique (SIT), as part of an integrated pest management strategy, we conducted radiation dose response studies. Late pupae of a *D. suzukii* laboratory strain was submitted to different radiation doses: 30, 50, 70, 80, 90, 100 and 120 Gy in a Cobalt-60 GammaCell 220 and we assessed following parameters: percent pupal emergence, percent adult malformations, adult male and female longevity, fecundity, percent egg hatching and number of F_1 progeny produced.

Rsults: Preliminary results indicate that irradiation doses had no effect on adult emergence, malformations and longevity. When irradiated above to 50 Gy, female fecundity was greatly reduced. Eggs of untreated females, mated with irradiated males, had lowest hatching success when the dose was above to 80 Gy.

Conclusion: These results will permit to identify a range of doses to pursue experiments on competitiveness of irradiated males.

Abstract ID 192

Continuing Practices: the Researches and Applications of Molecular Identification on Economically Important Fruit Flies in China

Zhihong LI, Fan JIANG and Yue ZHANG

China Agricultural University, Beijing Shi, China, Email: *lizh@cau.edu.cn*

Background: With the development of global trade and globalization, the economically important fruit flies have been introduced and spread more frequently and widely. The rapid and accurate identification of species is significant for the prevention and control of this kind of insect pests. However, it is difficult to accomplish the species identification of economically important fruit flies based on traditional methods and techniques of morphological characteristics, especially for the samples of eggs, larvae and pupae that are intercepted at ports of entry.

Continuing research on the molecular identification of economically important fruit flies and progress on its application is being made in recent decades in China.

Methodology: The technical system of molecular identification on economically important fruit flies, including DNA barcoding, PCR, real-time PCR and microfluidic dynamic array, was developed and applied systematically based on DNA barcodes of mtDNA COI.

Results: The technical system in this study could realize the species identification of 180 economically important fruit flies based on DNA barcoding and 27 species of economically important fruit flies in 6 genera (*Anastrepha*, *Bactrocera*, *Carpomya*, *Ceratitis*, *Dacus* and *Rhagoletis*, which received more attention by China) based on PCR, real-time PCR and microfluidic dynamic array. The above methods and techniques were applied successfully to identify unknown immature samples from port interceptions and field monitoring in China.

Conclusion: This molecular system provides technical support for rapid and accurate identification in the prevention and control of economically important fruit flies in China. It has broad potential as important reference under International Standards of Phytosanitary Measures (ISPM) for species detection and diagnosis of economically important fruit flies.

Key words: economically important fruit flies, molecular identification, DNA barcoding, PCR, real-time PCR, microfluidic dynamic array

Abstract ID 204

Exposure of Insect Cells to Ionizing Radiation *In Vivo* Induces Persistent Phosphorylation of a H2AX Homolog (H2avB)

Mohammad SIDDIQUI

Macquarie University, Australia, Email: *mohammad.siddiqui@mq.edu.au*

Background: The response of eukaryotic cells to ionizing radiation (IR)-induced double strand DNA breaks is highly conserved and involves a DNA repair mechanism characterized by the early phosphorylation of histone protein H2AX (producing the active form γH2AX). Although the expression of an induced γH2AX variant has been detected in *Drosophila melanogaster*, the expression and radiation response of a γH2AX homolog has not been reported in economically important fruit flies.

Methodology: We used *Bactrocera tryoni* (Diptera: Tephritidae, Queensland fruit fly, to investigate this response with a view to developing molecular assays to detect/quantify exposure of fruit flies to ionizing radiation and consequent DNA damage.

Results: Deep sequencing confirmed the presence of a H2AX homolog that we have termed H2AvB (and has an identical sequence to a histone reported from the human disease vector *Glossina morsitans*). A linear dose-response of γH2AvB (0–400 Gy ionizing radiation) was observed in whole *B. tryoni* pupal lysates 24 h post- ionizing radiation and was detected at doses as low as 20 Gy.

γH2AvB signal peaked at approximately 20 min after ionizing radiation exposure and at 24 h post-ionizing radiation the signal remained elevated but declined significantly by 5 days. Persistent and dose-dependent γH2AvB signal could be detected and quantified either by Western blot or laser scanning cytometry up to 17 days post-ionizing radiation exposure in histone extracts or isolated nuclei from adult *B. tryoni* flies (irradiated as pupae).

Conclusion: We conclude that ionizing radiation exposure in *B. tryoni* fly leads to persistent γH2AvB signals (over a period of days) that can easily be detected in irradiated insects (for example in shipped fruit after post-harvest irradiation) by Western blot or quantitative immunohistochemistry techniques.

These approaches have potential as the basis for assays for detection and quantification ionizing radiation exposure in pest fruit flies.

Abstract ID 216

The presence of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in the Cape Verde Archipelago

David HORTA-LOPES[1], Reinaldo PIMENTEL[2], Raimundo CABRERA-PÉREZ[3] and Alfesene BALDÉ[4]

[1]Departamento de Ciências e Engenharia do Ambiente - Universidade dos Açores, Ponta Delgada, Portugal, Email: *david.jh.lopes@uac.pt*
[2]CE3C – Centre for Ecology, Evolution and Environmental Changes, Azorean Biodiversity Group
[3]UDI Fitopatologia, Universidad de La Laguna, Laguna, Tenerife, Islas Canarias, Spain
[4]Instituto Nacional de Investigação e Desenvolvimento Agrário (INIDA), S. Jorge dos Órgãos, Ilha de Santiago, Cabo Verde

Background: Since the first report about the establishment of a new exotic fruit fly, back in 2003, in the Coast Province of Kenya, many European countries started surveys to determine its presence within their boundaries. In 2007, *Bactrocera dorsalis* Hendel started to appear in Cape Verde fruit production and only in 2011, within the activities of the research project CabMedMac (MAC/3/A163), a Macaronesia-wide alert was issued through official channels.

Methodology: To monitor the *B. dorsalis* population densities, traps were hung in banana and mango orchards.

Results: The constant high amount of captures of this insect in the Cape Verde archipelago is really worrying, as it shows that it found very suitable conditions to sustain and increase its population density levels. Fruit infestation also indicates that this species does not only damage ripened fruits, but as well as the ones still in growing status like green bananas.

Conclusion: Considering the fruit hosts richness present in all the other Macaronesia archipelagos (Azores, Canaries and Madeira), the natural highly polyphagous nature of the Oriental fruit flies poses a serious threat to the fruit and horticultural production of all these islands. Despite the Macaronesia-wide alert issued, additional measures should be put in action to prevent its invasion to the other archipelagos in Macaronesia.

Abstract ID 230

Relationship between Population Fluctuation of Oriental Fruit Fly *Bactrocera dorsalis* Hendel and Abiotic Factors in Yezin, Myanmar

Nwe Nwe YIN

Department of Agricultural Research, Ministry of Agriculture, Livestock and Irrigation, Nay Pyi Taw, Myanmar, Email: *nnyin86@gmail.com*

Background: In Myanmar, the Oriental fruit fly, *Bactrocera dorsalis* Hendel, is widely distributed and a very important pest of mango, citrus, guava and cashew (Morris and Waterhouse 1998). The *B. dorsalis species* complex can be found in Myanmar as an economically important group of pests (Drew and Hancock 1994). The abundance of larval hosts is one of the major factors regulating fruit fly populations (Kapatos and Fletcher 1984). Climate, particularly temperature and rainfall, is another major factor influencing the distribution of the fly.

It is very difficult to control the pest simply through the application of chemical pesticides due to their peculiar biological features. The objectives of this study were to monitor the population fluctuation patterns of *Bactrocera dorsalis*, and to determine the major factors affecting the population incidence in Yezin area.

Methodology: Traps were handmade and constructed based on the Steiner trap. Three Steiner traps, more than 50 m apart, were placed on fruit trees at a height of 2 m above the ground at each site. A pheromone lure was suspended inside each trap near the centre. The lure consisted of a small cotton ball soaked with 2 ml of methyl-eugenol, which was replaced at one week interval throughout the year. Male flies were attracted by the lures when they touched the cotton ball. *B. dorsalis* flies in each trap were identified and counted weekly starting from May, 2011 to December, 2011 and May, 2016 to December, 2016 in mango orchard farms of the Department of Agricultural Research in Yezin, Myanmar. The meteorological data were recorded from Yezin meteorological station in Department of Agricultural Research. Population data were analysed with meteorological data including temperature, duration of sunshine, and rainfall.

Results: In 2011, the highest mean number of male Oriental fruit flies/trap/day (241.42 ± 11.79) was recorded in June and the lowest (2.21 ± 0.40) in December. In 2016, the highest mean number of male Oriental fruit flies/trap/day (388.95 ± 8.56) was recorded in the month of June and the lowest (2.33 ± 0.20) in December.

Conclusion: Population fluctuation of male fruit flies was clearly observed to be positively correlated with temperature and rainfall, and negatively correlated with the duration of sunshine.

Key words: Oriental fruit fly, *Bactrocera dorsalis*, population fluctuation, mango orchard

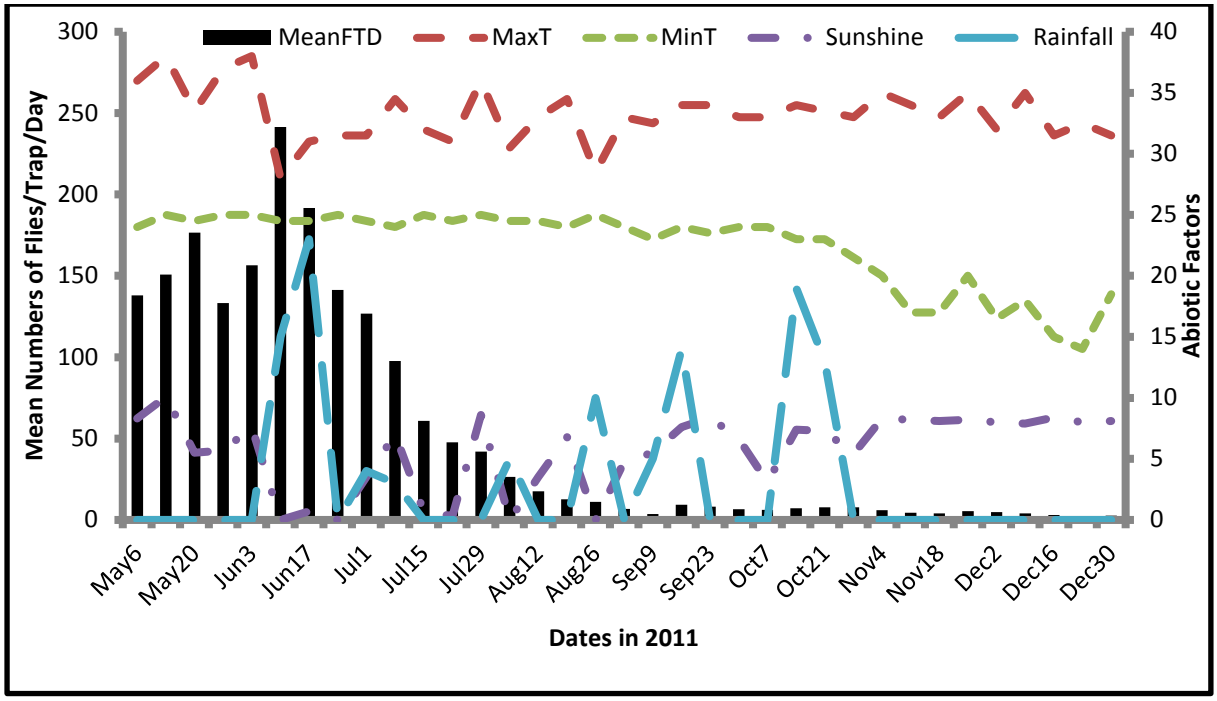


Fig. 1. *Relationship between mean number of flies/trap/day and abiotic factors in 2011.*

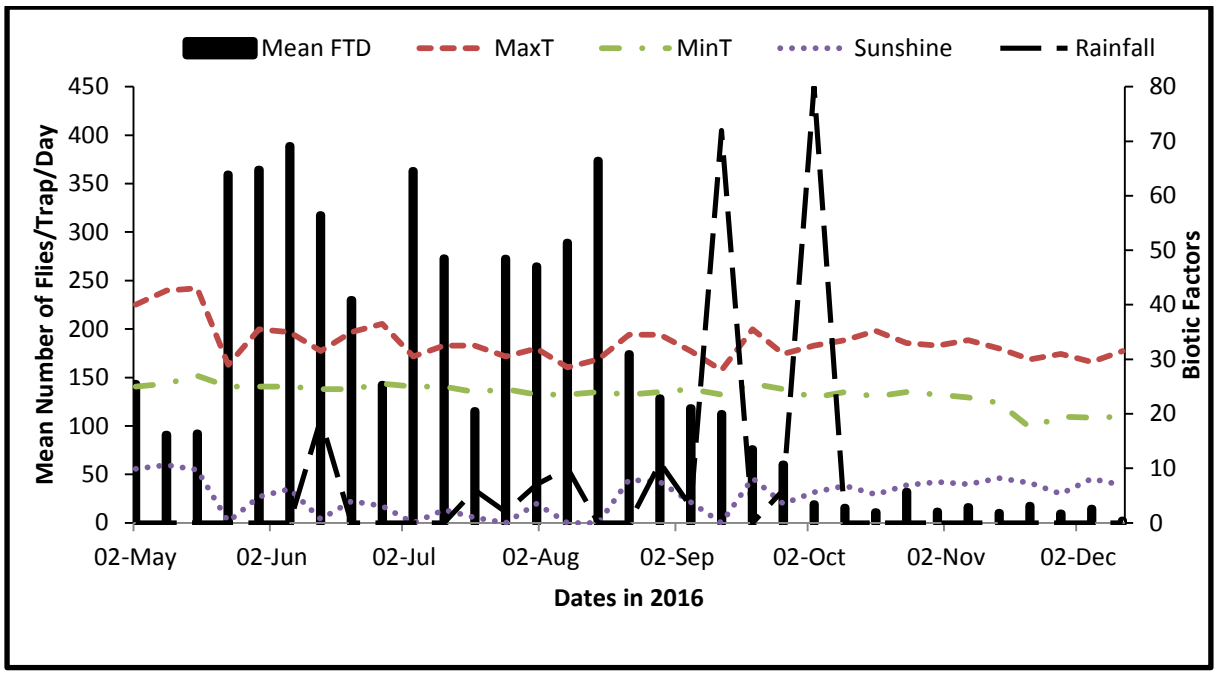


Fig. 2. Relationship between mean number of flies/trap/day and abiotic factors in 2016.

Poster Presentations Session 6: New Developments and Tools for Area-wide Integrated Pest Management

Abstract ID 5

Effect of Some Volatile Oils and/or Gamma Irradiation on the Reproduction of the Greater Wax Moth, *Galleria mellonella* by Using Contact Method

Hussein MOHAMED and Samira EL-NAGGAR

Egyptian Atomic Energy Authority, Nuclear Research Center, Cairo, Egypt, Email: *husseinmohamed29@yahoo.com*

Background: The greater wax moth, *Galleria mellonella* L., is a major pest of the honey bee, *Apis mellifera* L. It feeds on wax and pollen stored in the combs of active honey bee colonies. The extensive use of the synthetic insecticides leads to the biological imbalance due to the destruction of beneficial species such as parasitoids and predators of the pests, beside the destruction of pollinating insects such as honey bees.

Methodology: This study aimed at studying the effect of essential oils of five plants, namely; Peppermint, Marjoram, Geranium, Lemon grass, *Cymbopogon proxiomus* and Basil, *Ocimum basilicum*, were tested by the concentrations 0.625, 1.25, 2.50 and 5.00% on fecundity, fertility, some biological aspects, larval and pupal period and adult longevity of the greater wax moth, *G. mellonella*. It also aimed at studying the combined effect of gamma radiation with the doses 0,100 and 150 Gy and the essential oils on the biological aspects of the insect.

Results: There was a highly significant reduction in the average number of eggs laid per female *G. mellonella*, increasing with the concentrations of the tested volatile oils at all treatments. The percentage of egg hatch was also gradually but significantly decreased with increasing concentrations of all tested oils. Treatments at higher concentrations exhibited significant severe reduction in the egg hatch.

The percentage of the population significantly decreased with increasing concentration at most treatments. Adult emergence and sex ratio were unmatched with the dose increasing. The survival percentage was inversely correlated with the increase in concentration.

Conclusion: Data showed that the average *G. mellonella* larval and pupal period significantly increased among all concentrations at all treatments compared with the control treatment. The adult longevity of the unmated or mated males was longer than females at all treatments.

Key words: *Gamma radiation, essential oils,* Galleria mellonella, *fecundity, fertility, biological aspects*

Abstract ID 11

Gamma Radiation Dose to Generate Inherited Sterility in Tomato Leaf Miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)

María Verónica YUSEF[1], Mariana Mabel VISCARRET[2], Silvia Noemí LÓPEZ[2], Severino Carlos MICHELIN[3], Herlinda Luciana BRITOS[1], Antonio SÓLIS[1], Eva Sandra PAWLAK[1] and Mariana MALTER-TERRADA[1]

[1]Comisión Nacional de Energía Atómica, Argentina, Email: *mvyusef@gmail.com*
[2]Instituto Nacional de Tecnología Agropecuaria, Castelar, Argentina
[3]Autoridad Regulatoria Nuclear, Argentina

Background: The tomato moth, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a pest of South American origin, widespread throughout the tomato (*Solanum lycopersicum* Linnaeus) production areas in most of South American countries. It has been reported in many countries of Europe and also in the Mediterranean basin.

The Sterile Insect Technique (SIT) is a method for pest control of low environmental impact, which uses ionizing radiation. A variation of the SIT is called Inherited Sterility.

Methodology: The aim of this study was to determine the most suitable dose range of gamma radiation for the implementation of Inherited Sterility to control *T. absoluta*. The effect of 50, 75, 100, 120, 130, 150, 200, 250 and 300 Gy on pupae of both sexes was analysed under controlled laboratory conditions.

Results: Adult emergence decreased with increasing radiation dose. At doses above 200 Gy, deformed wings and bent legs were observed, making individuals unfit for mating. In order to establish the effects of radiation (50, 75, 100, 120, 130, 150 and 200 Gy) for use of Inherited Sterility, the following parameters were evaluated: fertility, fecundity, longevity and sex ratio of the F_1 generation of males and females irradiated in the pupae stage.

When irradiated males were crossed with untreated females, the fertility decreased with increasing doses applied. However, the longevity of irradiated insects was not affected by the different doses. In the dose range between 120 and 130 Gy, a reduction of fecundity and fertility of the F_1 was observed. When irradiated females with 100 Gy were crossed with untreated males, full sterility was observed.

Conclusion: A dose range of 120-130 Gy is suitable to generate Inherited Sterility in *T. absoluta*. Studies of morphological sperm packets are in progress in order to establish a methodology to differentiate the irradiated from the wild moths.

Abstract ID 13

A New and Very Potent Male Attractant for the Guava Fruit Fly, *Bactrocera correcta* (Diptera: Tephritidae)

Suk Ling WEE[1], Suksom CHINVINIJKUL[2], Keng Hong TAN[3] and Nishida RITSUO[4]

[1]Universiti Kebangsaan Malaysia, Selangor, Malaysia, Email: *slwee@ukm.edu.my*
[2]Department of Agriculture Extension, Bangkok, Thailand
[3]Tan Hak Heng Co., Johor Bahru, Malaysia
[4]Kyoto University, Japan

Background: The guava fruit fly, *Bactrocera correcta*, is an economically important key pest after the oriental fruit fly, *B. dorsalis*, in Thailand, Vietnam and China. Males of *B. correcta* are known to be attracted to and feed on methyl-eugenol (ME), a potent male attractant for many *Bactrocera* species. Our previous study has shown that the rectal gland of wild *B. correcta* males contains large quantities of sesquiterpenes, especially β-caryophyllene (CP) apart from two ME metabolites.

Methods: We conducted a) behavioural bioassays by comparing the age response of wildish *B. correcta* males to both CP and ME; and b) simultaneous field trapping using Latin Square designs for both attractants in Pathumthani suburb of Thailand where both *B. dorsalis* and *B. correcta* are known to co-exist. Age-related response to the attractants was also conducted to determine the basis of fly physiological readiness for attractant response during sexual maturation.

Results: Males of *B. correcta* responded to CP at a much younger age prior to sexual maturation than ME. Field trappings revealed interesting findings in that ME-baited traps captured both *B. dorsalis* and *B. correcta*, while CP-baited traps only B. correcta. Furthermore, the numbers of *B. correcta* males captured in CP-baited traps are significantly higher than that in ME-baited traps.

Conclusion: *B. correcta* males responded to CP at a much younger age prior to sexual maturity than the commonly used ME attractant. This study confirms that CP is a more potent male lure than ME for the guava fruit fly. Thereby, the discovery represents a significant input in improving the current tools and technology used in monitoring, detection and control of fruit fly pests, specifically for the guava fruit fly, which is a potentially and highly invasive species due to its polyphagous nature.

Abstract ID 17

Utilizing Loci Linked to Genetic Sexing Traits to Develop a Robust Diagnostic Assay for Discriminating a *Bactrocera cucurbitae* Genetic Sexing Strain from Wild Individuals

Sheina SIM and Scott GEIB

USDA-ARS, Hilo, Hawaii, USA, Email: *ssim8@hawaii.edu*

The melon fly *Bactrocera cucurbitae* (Coquillett), is a destructive agricultural pest and is among one of the many species that is the target of expensive population suppression and eradication efforts within state and federal departments of agriculture. Area-wide integrated pest management programmes control some species such as the Mediterranean fruit fly and the Mexican fruit fly through the release of sterile males known as the sterile insect technique (SIT).

This method is not currently employed for *B. cucurbitae* in the United States, though a genetic sexing strain (GSS) with a sexually dimorphic pupal colour morph that can be used for mass-rearing and release exists for the melon fly. In the advent of its release, a diagnostic tool will be necessary to differentiate between wild and released sterile melon flies to prevent unnecessary and costly quarantines. The purpose of this project was to identify SNP loci tightly linked to the causative mutation of the genetic sexing trait white pupae (*wp*) in the genetic sexing strain of *B. cucurbitae*.

To do so, a QTL map was generated using a mapping population and SNPs obtained through double-digest restriction associated DNA sequencing (ddRAD-seq). Primers were then designed which flank the most tightly linked SNPs as well as non-linked loci which demonstrated a tight association to the GSS colony and targets were successful in differentiating between GSS colony and wild flies. Tightly linked loci were further interrogated using low-coverage resequencing to identify the putative genes governing the pupal colour trait.

These data can be used to develop a genetic assay for differentiating between recaptured sterile males and wild males. This will be useful for the improvement of the melon fly GSS colony and can be used to identify an orthologous gene in other species to create novel genetic sexing strains.

Abstract ID 19

Evaluation of Two Trap Types on the Capture of Fruit Flies (Diptera: Tephritidae) in the Assin North Municipality, Ghana

Enoch Selorm Kofi OFORI[1], Samuel Abukari BAWA[2], and Maxwell BILLAH[3]

[1]Ghana Atomic Energy Commission, Ghana, Email: *selormofori@yahoo.com*
[2]Asuansi Agricultural Research Station, Ghana
[3]Department of Animal Biology and Conservation Science, University of Ghana

Introduction: True fruit flies (Diptera: Tephritidae) pose a major threat to the horticultural industry worldwide and Ghana is no exception. Fruit flies are one of the major contributing factors to crop losses in countries where they are found. The Oriental fruit fly, *Bactrocera dorsalis* Hendel is one of the recently introduced fruit fly species in Africa. It was first detected in Kenya in 2003 and in Ghana in 2005. The insect is now well established in Ghana, causing extensive damage to fruits like citrus, mango, avocado etc. The insect is also competing with other endemic fruit fly species such as *Ceratitis cosyra* Walker, *C. capitata* Wiedemann and *C. ditissima* (Munro). Although *B. dorsalis* is very important in mango production, there is evidence that it can also damage other fruit crops. *B. dorsalis* is reported to be the most destructive among both the indigenous and exotic species, and it is responsible for the extensive economic losses seen throughout sub-Saharan Africa with damage levels exceeding 80%. In Assin North Municipality, citrus fruit losses due to fruit fly infestation are estimated at 45-50%. Several control measures against fruit flies have been developed, including chemical control among others. Some of the most widely used control measures which pose little or no harm to the environment is the use of traps, lures and baits. The success of the mass-trapping strategy depends on the efficiency of traps and lures. Trap designs including different colours and shapes can influence efficacy in fruit fly catches.

Objective: Here we evaluate the effectiveness of the Ecoman and Mineral Water Bottle traps baited with methyl-eugenol and insecticide on trapping fruit flies in an orchard in Ghana. We compared the two traps to better advice local citrus farmers in the Assin North municipality as to which trap to use in their orchards for effective fruit fly monitoring.

Methodology: The study was conducted at Akonfudi in the Assin North Municipality in the Central Region of Ghana from September 2014 to October 2014. The study plot (1.5 acres) was divided into three subplots that contained all the traps and treatments (traps with and without servicing) evaluated. Both traps were baited with methyl- eugenol with an insecticide, Deltamethrin, 25g active ingredient/L in the ratio of 4:1.

Results: Ecoman traps were more efficient in catching *B. dorsalis* and *C. ditissima* compared with mineral water bottles, even when traps were not serviced.

Conclusion: It is recommended that for effective trapping of *B. dorsalis* and *C. ditissima*, Ecoman traps can be used.

Abstract ID 20

Characterization of Genetic Sexing Traits in Established Mass-rearing Lines for Transfer to Economically Important Tephritid Fruit Fly Species

Scott GEIB and Sheina SIM

USDA-ARS, Hilo, Hawaii, USA, Email: *scott.geib@ars.usda.gov*

Tephritid fruit flies (Diptera: Tephritidae) are major pests of worldwide economic importance. Their threat to high value agriculture is so extensive that area-wide integrated pest management programmes (AW-IPM) are employed to prevent their long-term establishment in the mainland United States.

An important component of these AW-IPM programmes is the use of the sterile insect technique (SIT) by taking advantage of genetic sexing strains (GSS) that are established for these species. In order to have an effective SIT programme, a genetic sexing strain (GSS) needs to be established for the species of interest, which facilitates cost-effective and rapid separation of female and male flies during mass-rearing, and releases of male-only sterile flies.

Our goal is to identify the genetic basis for the white pupae and temperature sensitive lethal traits in existing GSS, identify the causative mutations in genes causing phenotypic changes in these strains, and develop tools to transfer these traits to other economically important Tephritid species using targeted genome editing approaches. To address these objectives, we employed methodologies that bring together classical genetics with high-throughput sequencing techniques and emerging genome editing technologies.

Once the causative mutations are identified, through comparative genomic analysis (orthology/synteny) to other Tephritid genomes, putative targets in those genomes will be identified to introduce the same GSS mutations into colony lines of those species. This will provide the foundation to developing GSS lines for important Tephritid pests which currently are not amenable to male-only mass-rearing for SIT.

Abstract ID 21

Characterization of Bacterial Endosymbionts Associated with Leafhopper *Amrasca biguttula biguttula* and Their Role in Imparting Insecticide Resistance

Gopalsamy SIVAKUMAR

ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India, Email: *sivakumarg.nbaii@gmail.com*

Background: Leafhopper *Amrasca biguttula biguttula* is one of the serious insect pests of cotton, okra, sunflower, bhendi, brinjal and causing substantial yield loss. Farmers generally spray 10 to 15 rounds of different pesticides during the crop season against leafhopper. The reprints suggest that the leafhopper has developed resistance to many recommended insecticides. Despite several rounds of sprays, the leafhoppers continue to breed on many crops and often control failures are reported, thus causing significant yield loss. The field observations followed by toxicity studies against different insecticides confirm that the pest has developed resistance.

Recent line of work at global level suggest that gut bacterial symbionts play an important role in many physiological functions of the insects, including detoxification of noxious compounds including insecticides. In light of above the present work was carried out to characterize the diversity of culturable gut bacterial symbionts and determine their role as endosymbionts in *A. biguttula biguttula* in insecticide degradation, thus imparting insecticide resistance. Interactions between the insects and their gut bacterial endosymbionts may provide the way for novel approaches for insect pests control. No attempts have been made so far across the world to study the bacterial endosymbionts associated with *A. biguttula biguttula*.

Methodology: Sixteen populations of *A. biguttula biguttula* were collected from insecticide sprayed fields of cotton from various locations of India. The microflora from *A. biguttula biguttula* were isolated from the gut as per the standard protocol and characterized through morphological parameters and 16S rRNA gene sequences.

Results: Thirty culturable gut bacterial endosymbionts were found associated with the sixteen field populations of leafhopper and were identified through 16S rRNA gene sequences, which revealed that the sequences of the organisms of field collected population showed a 97-100% similarity with the available database at GenBank, NCBI. A good diversity of microflora was recorded across the locations and they are unique with respect to each location.

The major gut bacterial genera characterized were *Staphylococcus, Enterococcus, Bacillus, Enterobacter, Serratia, Lysinibacillus, Proteus, Methylobacterium, Pseudomonas,*

Stenotrophomonas, Staphylococcus, Brevibacterium, Microbacterium, Phenylbacterium and *Paenibacillus*.

The present study proved that the occurrence of high number of bacterial diversity associated with the insects exposed to heavy insecticides might be responsible for imparting resistance to the insects against insecticides. Two bacterial symbionts, *Enterobacter cloacae* and *Bacillus pumilus* were taken to further studies for their role in insecticide resistance. The present study confirmed the role of these two bacterial symbionts in insecticide degradation.

Maximum growth of *E. cloacae* and *B. pumilus* was recorded when these organisms were grown in minimal broth which was amended with various concentration of insecticides acephate and imidacloprid. The peaks of liquid chromatography mass-spectrometry (LCMS) analysis indicated the ability of *E. cloacae* and *B. pumilus* to degrade Acephate (183.78) into Methamidophos (142.2). Further esterase, an important enzyme involved in insecticide degradation was also detected from the *B. pumilus* and *E. cloacae*.

Conclusion: The present study revealed the diversity of the bacterial endosymbionts associated with leafhopper and pinpointed the exact role played by the bacteria in insecticide degradation thus imparting insecticide resistance.

Abstract ID 22

Development and Reproduction of *Trichogramma cacoeciae* on *Ephestia kuehniella* Eggs: Important Factors to Consider

Mohammed MANSOUR

Atomic Energy Commission of Syria, Damascus, Syria

Background: The codling moth, *Cydia pomonella* (L.), is a key pest of apple orchards in Syria. Chemical control of this pest has many drawbacks including insecticide resistance, reduced natural enemies, disruption of beneficial species and creation of secondary phytophagous mite problems. An integrated approach using non-insecticidal methods (pheromones, orchard sanitation, agricultural practices, etc.) and natural enemies, particularly parasitoids, to control this pest in apple orchards is seriously considered. An egg parasitoid, *Trichogramma cacoeciae*, is common on codling moth eggs in neglected orchards and is suggested as a biological control agent in commercial orchards. Augmentative releases of this species in apple orchards require mass-rearing on a large-scale.

Methodology: Eggs of the Mediterranean flour moth, *Ephestia kuehniella*, are often used as an alternative host to rear *Trichogramma* species as rearing on natural hosts can be very costly. Rearing *T. cacoeciae* on *E. kuehniella* eggs requires sufficient knowledge of the factors affecting reproduction of this parasitoid, particularly temperature, food and host age and availability. Rearing *T. cacoeciae* was done in a controlled environmental cabinet at 25±1 ºC, 60-70 % RH and 12 h-photophase. Effects of temperature (between 15 and 35± 1 ºC) on *T. cacoeciae* parasitization rate, longevity, survival of immatures to the adult stage and rate of development were examined. In addition, the effects of food and host availability on parasitoid longevity and its parasitization ability were studied. Furthermore, the effects of host age (age of *E. kuehniella* eggs) on its suitability for parasitzation by *T. cacoeciae* females, and parasitoid age on its ability to parasitize *E. kuehniella* eggs were investigated.

Results: Results showed that temperature had a clear effect on the mean number of parasitized eggs/female and survival of immatures to the adult stage. Parasitization was highest in the 1st day after emergence, continued for about 10 days with gradual decrease and was best at 25±1 ºC (see Figure 1). Results also showed an inverse relationship between temperature and mean adult longevity; longevity decreased with increasing temperature. The availability of food and hosts increased adult parasitization and longevity, and the younger the host (*E. kuehniella* eggs) the more it was suitable for parasitism.

Conclusion: In summary, *T. cacoeciae* can develop and reproduce efficiently on *E. kuehniella* eggs at temperatures between 20 and 30^{o}C. The younger the eggs, the more they are suitable for parasitization, and providing food has a positive effect on the parasitoid longevity and reproduction ability.

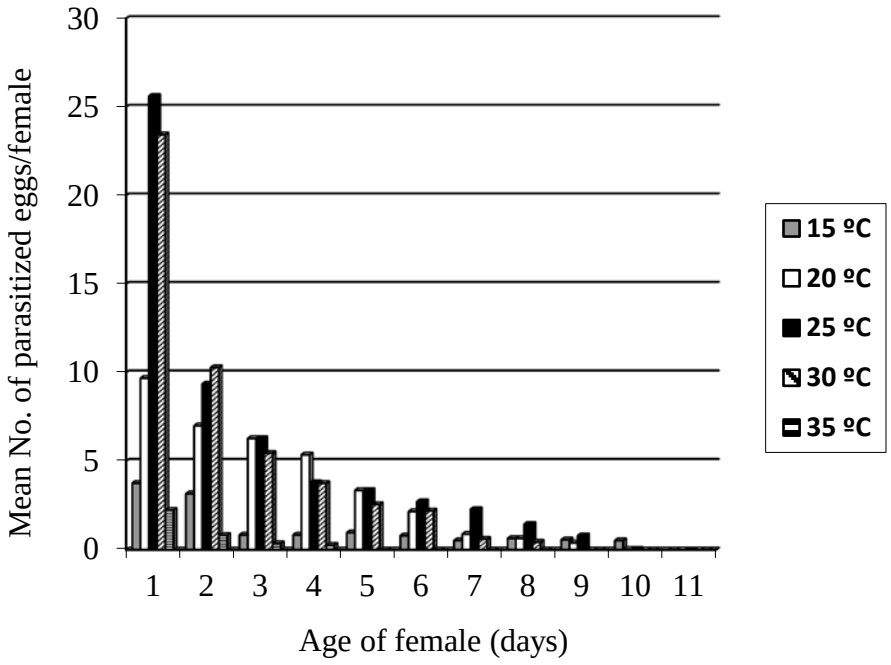


Fig.1. *Effects of age of T. cacoeciae on mean daily egg production at different temperatures.*

Abstract ID 31

Symbiont Diversity and Feeding Strategies in Insect Agricultural Pests

Maarten DE COCK[1], Massimiliano VIRGILIO[1], Anne WILLEMS[2], Peter VANDAMME[2], Antonis AUGUSTINOS[3], Alessandro DE SCISCIO[1], Luis BOTA[4], Kostas BOURTZIS[5], Domingos CUGALA[6], Hélène DELATTE[7], Aruna MANRAKHAN[8], Nikos PAPADOPOULOS[9], Chris WELDON[10] and Marc DE MEYER[1]

[1]Invertebrates Unit, Royal Museum for Central-Africa, Tervuren, Belgium, Email: *maarten.de.cock@africamuseum.be*
[2]Laboratory of Microbiology, University of Gent, Ghent, Belgium
[3]University of Patras, Agrinio, Greece
[4]Direcção Provincial de Agricultura de Manica, Chimoio, Mozambique
[5]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria
[6]Eduoardo Mondlane University, Maputo, Mozambique
[7]CIRAD, St Pierre, La Réunion
[8]Citrus Research International, Nelspruit, South Africa
[9]University of Thessaly, Volos, Greece
[10]University of Pretoria, Pretoria, South Africa

Gut microbionts play a fundamental ecological and evolutionary role in phytophagous insects by making the plant tissue edible and by promoting adaptation between insects and host plants. SYMDIV is a collaborative project between the Royal Museum for Central Africa (Tervuren, BE) and the Laboratory of Microbiology of the University of Ghent funded by the Belgian Science Policy (BELSPO).

This project, consisting of four work packages (WPs), aims at describing and comparing the gut microbiomes of phytophagous tephritid flies (Diptera, Tephritidae) with different feeding strategies (from strictly monophagous to highly polyphagous). In particular we aim at (a) comparing the 16S microbiome profiles of species such as *Bactrocera dorsalis*, *Ceratitis capitata*, etc., across multiple African and Mediterranean locations and across different host plants (WP1 and WP2) (b) cultivating and isolating selected microbial strains (WP3) and (c) compiling and publishing a database of endosymbionts – host associations (WP4).

A pilot methodological test is currently ongoing in collaboration with FAO/IAEA, with the objective of optimising the experimental and analytical protocols. The SYMDIV project will provide baseline data to better understand relationship between gut microbionts, host range spectrum and metabolic processes in tephritid fruit flies. We will also isolate and characterise bacterial strains that could be tested as probiotics for SIT mass-rearing.

Abstract ID 33

Efficient, User- and Environment-Friendly Protein Bait Trap to Replace Spot Application in Area-Wide Management of Fruit Flies

Nimal Premaratne Bandara KASTURI ARACHCHILAGE[1], Sujeewa Kumari LIYANAGEDARA[2], Shyama Pushpakumari ALUTHGE[3] and Nilupa Eranganie Mahagollage MAHAGOLLAGE[3]

[1]Lanka Agroartis (Pvt) Ltd., Pilimatalawa, Sri Lanka, Email: *premaratnebandara@yahoo.com*
[2]Horticulture Crop Research and Development Institute, Department of Agriculture
[3]Fruit Research and Development Institute

Background: Of the 35 recorded species of fruit flies in Sri Lanka, seven cause economic damage to cultivated fruits and vegetables. Diversified agro-ecosystems with small-scale cultivation facilitate continuous supply of food and shelter for multiplication of fruit fly pest.

Spot application of locally produced protein bait (LakGro Bait®) played a major role since 2009 in the Department of Agriculture's area-wide integrated fruit fly management package, which successfully controlled damage with the produce routinely meeting export standards. However, rapid loss of bait from leaf surfaces in adverse weather conditions and fungal contamination meant frequent and repeated application was required. This drawback underlined the need for a more efficient application technique.

Methodology: Laboratory, green house, screen cage and field experiments were carried out at the Horticulture Crop Research and Development Institute (HORDI) to develop a more efficient application technique for protein bait against melon fly, *Bactrocera cucurbitae*. Various trap models loaded with protein bait (LakGro Bait®) were evaluated for their attractiveness, effective duration and efficiency in use of protein bait and its associated insecticide, spinosad. Successful trap models were evaluated in research fields at HORDI and Fruit Research and Development Institute (FRDI) and in pilot scale farmer fields during three cultivation seasons (2015-2016). Experiments were in Randomized Complete Block Design with 4 or more replications and data were analysed using SAS software by CATMOD procedure.

Results: The results of the laboratory experiments indicated that the protein bait trap model (Figure 1) loaded with undiluted LakGro Bait® mixed with spinosad at 5 ppm (A.I.) was highly attractive to melon fly with activity persisting for 14 days under field conditions without a significant drop ($p=0.05$). Research field evaluations showed that both protein bait trap and spot application were equally effective for controlling fruit fly.

The trap consisted of a PVC ring (90 mm diameter), dorsal thickness 60 mm and ventral thickness 40 mm hung in the field with the help metal wire and a sponge plate 25 mm thickness is inserted into the ring. Protein bait (LakGro bait mixed with spinosad 5 ppm AI) is applied on the sponge plate in a circle (5 ml/trap) using a delivery device.

The recommended number of traps per ha is 200-400 and replacement time was 10-14 days under local field conditions. The other side of the sponge could be used for the next application and the plate is re-usable after thorough rinsing with water. The bait application under local conditions cost Rs. 2250-4500/ha/application.

Fig. 1. *The selected trap model loaded with undiluted protein bait (LakGro Bait®) mixed with spinosad (Success® 2.5 SC) at 5ppm (A.I.).*

Farmer acceptance studies indicated farmer preference for the protein bait trap over spot application due to its convenience, long lasting effectiveness irrespective of weather conditions, easy self-assembly, low cost (Rs. 8/trap) and reusability. The trap also reduced insecticides (spinosad) usage from 125-250 to 5-10 µl AI/ha.

Conclusion: Spot application of protein bait in area-wide integrated management of fruit fly pests can be advantageously replaced by the more efficient user friendly protein bait trap.

Abstract ID 35

Studying Stability and Mating Competitiveness of the Genetic Sexing Strain of Mediterranean Fruit Fly, *Ceratitis capitata* (Wied.)

Ahmed SHOMAN[1], Mohamed EL-SHEIKH[2], Salah ELNGAR[2], Samir IBRAHIM[1] and Waheed SAYED[1]

[1]Department of Biological Application, Nuclear Research Centre, Atomic Energy Authority, Cairo, Egypt, Email*: aashoman@hotmail.com*

[2]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt

Background: In facilities of mass-rearing genetic sexing strains (GSS), it is necessary to introduce specific rearing methodologies, which in view of the very small number of recombination events, enable stable and predictable mass-production of Mediterranean fruit fly males. This study aimed to assess the critical need to monitor the stability in both the mass- and filter-rearing, as well as the mating competitiveness of the V8-Sr2 strain.

Methodology: Stock cultures of Mediterranean fruit fly mutant strains were maintained for several generations in the laboratories of the Entomology Unit, Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt, with the cooperation of the FAO/IAEA Insect Pest Control Laboratory, Vienna, Austria. In the semi field cage test, mating competitiveness and sexual compatibility were evaluated following the International Fruit Fly Quality Control Manual (FAO/ IAEA/ USDA 2003).

Results: Stability of genetic sexing strain V8-Sr2 in mass- and filter-rearing. The results indicate that the mass-rearing of GSS V8-Sr2 males (brown pupae) was stable for up to 7 generations. In the F_8 generation, brown pupal colour showed 1% instability, with brown pupae producing females. The percentage of brown pupae females increased in the subsequent generations F_9, F_{10}, F_{11} and F_{12}, respectively. On the other hand, in case of filter-rearing, instability of brown pupae was observed in the F_8 and F_{11} generations, where the percentage of brown pupae females was (1%), while, the percentage of brown pupae was (100%) in the other generations. The same trend was observed in the accumulation of white pupae recombinants. The instability of white pupae females was recorded in the F_8 generation with a very low recombinant percentage, which increased in the F_9, F_{10}, F_{11} and F_{12}, respectively. The recombination of white pupae in females was observed in the F_8 generation of filter-rearing GSS V8-Sr 2 strain. However, the white pupae in males were not observed in the next successive generations.

The accumulation of the sergeant Sr2 recombinants in both the mass- and filter-rearing revealed that, in the mass-rearing treatment, the recombination started in the 9th generation (2%). The 2 abdominal stripes (wild type) of males were increased in the F_{10}, F_{11} and F_{12}, respectively. Regarding the filter-rearing system, the removal of recombinants to maintain

stability had an impact in reducing the percentage of males with the 2 abdominal stripes after the F_9 generation.

It is worth mentioning that the GSS males were competitive with BSS males for mating with both GSS and BSS females (Table 1). The females of BSS accepted both BSS and GSS males with similar mating success; however the BSS males had longer duration of mating than GSS males when both mated with BSS females.

Conclusion: According to the results, in facilities mass-rearing GSS V8- Sr2, it is necessary to introduce a filter-rearing colony (small closed colony) to improve the quality and enable stable and predictable mass-rearing.

Table 1. *Results of mating success (%), average mating duration and mating site (%) of GSS V8-4-sr2 and BSS C. capitata on a field-cage tree.*

Combination		Mating success (%)	Average mating duration (min.)	Mating site (%)	
				Tree	Net screen
BSS	♂ x	38.1 a	131.4 ± 9.5 a	96.4 a	3.6 a
BSS	♂ x (	13.0 b	117.1±11.2 b	94.0 a	6.0 a
GSS	♂ x	36.0 a	121.4±10.5 b	74.3 b	25.7 b
GSS	♂ x (	12.9 b	118.4± 9.9 b	83.3 c	16.7 c

Abstract ID 37

Molecular Diversity, Function of Gut Bacteria on Improving Ecological Fitness of Sterile Males in *Bactrocera dorsalis* Adult Flies

Zhaohui CAI, Zhichao YAO, Hongxiu WANG, Yushan LI and Hongyu ZHANG

State Key Laboratory of Agricultural Microbiology, Key Laboratory of Horticultural Plant Biology, Ministry of Education and Institute of Urban and Horticultural Entomology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China, Email: *hongyu.zhang@mail.hzau.edu.cn*

In this study, we investigate the structures and composition of the bacterial community in the adult gut of *Bactrocera dorsalis*. The results indicate that commensal bacterial phylotypes are Enterobacteriaceae, e.g. those related to *Klebsiella*, *Citrobacter*, *Enterobacter*, *Pectobacterium* and *Serratia*. The gut bacterial communities of *B. dorsalis* from the different populations studied were significantly different from each other. These results suggested that the *B. dorsalis* adult gut contains a diverse bacterial community with some stable commensal bacteria. Among them, feeding with the minor *Bacillus* had a negative effect on fly longevity, while feeding with the dominant Enterobacteriaceae mixture had a beneficial effect. Compared to flies fed on sugar alone, the average longevity of flies fed with *Bacillus* was significantly reduced. Conversely, flies fed with Enterobacteriaceae died more gradually and lived significantly longer than flies fed on sugar.

We examined the complex interactions among dosage of radiation-treatment, gut microbiota, and the host ecological fitness abilities. The results showed that irradiated male flies had significantly reduced ecological fitness abilities, like male mating ability, male feeding rate, flight distance and flight time compared with the unirradiated male group, and the gut bacterial community homeostasis has been disrupted in irradiated male flies. On the other hand, a group of irradiated males, fed with gut symbionts *Klebsiella* or *Citrobacter*, showed that all ecological fitness parameters of irradiated males can be restored to the level of the unirradiated males group.

This work was supported by the International Atomic Energy Agency's Coordinated Research Project (No. D42016), the National Natural Science Foundation of China (No. 31572008), the earmarked fund for the China Agricultural Research System (No. CARS-27) and Fundamental Research Funds for the Central Universities (No. 2014PY005).

Abstract ID 38

The Gut Bacterial Community Homeostasis Regulation of Oriental Fruit Fly (*Bactrocera dorsalis*)

Zhichao YAO, Ailin WANG, Yushan LI, Zhaohui CAI, Bruno LEMAITRE and Hongyu ZHANG

State Key Laboratory of Agricultural Microbiology, Key Laboratory of Horticultural Plant Biology, Ministry of Education and Institute of Urban and Horticultural Entomology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, People's Republic of China, Email: *cai_zhaohui@163.com*

The guts of metazoans are in permanent contact with the microbial realm that includes beneficial symbionts, nonsymbionts, food-borne microbes and life-threatening pathogens. However, little is known concerning how host immunity affects the gut bacterial community.

Here, we analyse the role of a dual oxidase gene (BdDuox) in regulating the intestinal bacterial community homeostasis of the oriental fruit fly, *Bactrocera dorsalis*. The results showed that knockdown of BdDuox led to an increased bacterial load, and to a decrease in the relative abundance of Enterobacteriaceae and Leuconostocaceae bacterial symbionts in the gut. The resulting dysbiosis, in turn, stimulated an immune response by activating BdDuox and promoting reactive oxygen species (ROS) production that regulates the composition and structure of the gut bacterial community to normal status by repressing the overgrowth of minor pathobionts.

Our results suggest that BdDuox plays a pivotal role in regulating the homeostasis of the gut bacterial community in *B. dorsalis*.

This work was supported by the National Natural Science Foundation of China (No. 31572008), the earmarked fund for the China Agricultural Research System (Nos. CARS- -27), International Atomic Energy Agency's Coordinated Research Project (No. D42016) and Fundamental Research Funds for the Central Universities (No. 2014PY005 and 2662015PY129).

Abstract ID 52

Development and Evaluation of an Automated Sterile Fruit Fly Dispersal System

Les ZELLER[1], Paul KAMEL[1], Bill WOODS[2], Ernie STEINER[2], Don MCINNIS[3], Brett RENTON[2] and Peter CRISP[4]

[1]National Centre for Engineering in Agriculture (NCEA), University of Southern Queensland, Toowoomba, Australia, Email: *les.zeller@usq.edu.au*
[2]Department of Agriculture and Food, Perth, Western Australia (DAFWA)
[3]US Department of Agriculture (Retired)
[4]South Australian Research and Development Institute (SARDI), Adelaide, Australia

Background: The Sterile Insect Technology (SIT) is the insect pest control method utilised in area-wide integrated pest management (AW-IPM) in South Australia for outbreaks of Queensland fruit fly (*Bactrocera tryoni*, QFF) and Mediterranean fruit flies (*Ceratitis capitata*). The SIT is also used in Western Australia to control the Mediterranean fruit fly. Previous methods of sterile fly release in Australia have been ground-based and have utilised a predominantly manual process.

This paper describes the development and evaluation of an automated, mobile, ground based sterile fruit fly release system that improves the efficiency, accuracy and safety of ground based mobile sterile insect releases into the field.

Methodology: The system was designed to be portable, operate from a 12 volts DC power source, provide chilled storage for flies and automatically release flies in predefined zones at predefined rates. The system incorporates a custom designed controller that utilises GPS technology to control the release rate of sterile flies relative to ground speed and location. The system also includes a refrigerated hopper unit and controls a heater system to revive the chilled fruit flies prior to release and a blower fan and ducting to disperse flies upwardly into the air.

Field and laboratory experiments were undertaken to determine the control system's ability to provide accurate and repeatable metering of chilled fruit flies over a range of ground speeds and fly release rates.

Results: Results of field releases as part of a Mediterranean fruit fly eradication programme in Carnarvon, Western Australia indicated:

• Operational safety and efficiencies have been improved by having a hands-free system with no labour component required other than driving the release vehicle. For the work in Western Australia this reduced the number of operational staff required from 3 to 1, improving efficiencies by 66%.

• As an auger is used to meter out sterile insects the accuracy for this system was determined by quantifying the volume of flies per auger revolution (16.24g with a SD of 1.7g), fly density, e.g. flies per volume (40 flies/ml, SD 8 flies/ml), mass of chilled flies, e.g. flies per gram (160 flies/g, SD 34 flies/g) and the ability for the control system to regulate the auger speed over a range of ground speeds and fly release rates. Data was collected from 3 sections of road at 3 different speeds and 3 different release rates to create an index for a relationship between these variables and auger revolutions (0.60 with an SD of 0.02).

• Fly distribution measurements during field testing in 2 zones using sticky traps showed catch rates 3.3 times higher in the zone where the release rate was doubled with flies well distributed over each zone.

Abstract ID 57

Vegetable Oil Supplements to Improve the Basic Larval Artificial Diet for the Mass-rearing of the European Grapevine Moth (Lepidoptera: Tortricidae)

George SAOUR

Atomic Energy Comission of Syria, Damascus, Syria, Email: *gsaour@aec.org.sy*

The European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae), is amongst the most distructive pests of grapes and berries in southern Europe and the Middle East. Studies to evaluate the effects of replacing the maize oil in *L. botrana* basic larval diet by either the grape seed oil or the ginger root oil with or without casein supplement, in order to make more nutritious and palatable diet for *L. botrana* larvae, were performed.

Larval and pupal mortality, larval and pupal developmental period, moth weight, no. of emerged moths, fecundity, moth longevity, and diet consumption per larvae differed significantly between the different artificial diets tested. In general, replacing maize oil with either grape seed or ginger root oils significantly improved, the quality of the produced moths (i.e. reduced the developmental time, increase the moth weight, adult longevity and female fecundity).

However, the highest values of moth quality were recorded when *L. botrana* larvae were reared on "casein + ginger oil" diet and the lowest from larvae reared on the basic diet supplemented by maize oil. Nutritional indices calculated for *L. botrana* varied significantly with the diet supplements. Host suitability, food consumption and growth rates were highest on "casein + ginger oil" diet. This suggests that the last diet is a better food source for larval growth and development than the other diets tested. Of the six different larval diets studied, the diet supplemented by maize oil was least suitable for *L. botrana* development as shown by the lowest host suitability, food consumption and growth rates.

The mean percentage of males that successfully flew up (moth flight ability) was related to the food supplements added to the larval diets. There were significant differences in male's flight ability among the different diets. Irrespective of the 4th day following the initiation of the experiment, the mean percentage of flyer males of ginger oil diet was significantly higher than that of the maize oil diet either with or without the addition of casein. In contrast, the mean percentage of non-flyer males emerging from larvae that were reared on ginger oil or "casein + ginger oil" diets was significantly lower than that emerged from the other diets tested.

The data of the current study will help to establish an efficient and effective diet for *L. botrana* mass-rearing, which is a fundamental component of a sterile insect technique / inherited sterility (SIT/IS) programme.

Abstract ID 58

Sperm Behaviour as a Key Tool Ensuring Operative Efficiency of Radio-genetic 'F_1 Sterility Technique' for Population Suppression of an Economically Serious Indian Pest, *Spodoptera litura* (Fabr.) (Lepidoptera: Noctuidae) in Laboratory and Field Simulated Cages

Rakesh Kumar SETH, Zubeda KHAN, Dev Kant RAO, Mahtab ZARIN and Ranjana SETH

Applied Entomology and Radiation Biology Unit, Department of Zoology, University of Delhi, Delhi, India, Email: *rkseth57@gmail.com*

Background: Sperm activity, as a pivotal manifestation of mating competiveness, is a crucial attribute of viability and reproductive competence of sub-sterilized male moths to be employed in radio-genetic 'Inherited (F_1) Sterility Technique' for suppression of a noctuid tropical pest, *Spodoptera litura* (Fabr.).

Methodology: Various parameters pertaining to sperm dynamics, viz. sperm production, sperm descent circadian rhythm, sperm activation, sperm motility in males and in females during and after mating, amphimixis, chromosomal aberrations in spermatocytes and sperm utilization pattern, along with mating competitiveness of *S. litura* were evaluated in parent (P) males irradiated with a sub-sterilizing gamma dose of 130 Gy and in their F_1 generation males in laboratory and field simulated cages.

Results: Production of eupyrene and apyrene spermatozoa in the testes and the rhythmic cycles of sperm descent from the testes to the upper vas deferens (UVD), seminal vesicle (SV) and duplex were not significantly influenced in P moths at 130 Gy and their F_1 male moths. In vitro activation of apyrene spermatozoa was not diminished in irradiated P males at 130 Gy, while slight reduction was noticed in F_1 males derived from 130 Gy treated male parents. Mating status was not a pronounced factor in eliciting the motility of irradiated sperm. Further, the sperm activation of released irradiated (130 Gy) parent males and their F_1 progeny moths in the field cages was assessed to be quite similar to that of unirradiated moths (Figure1).

Sperm utilization pattern reflected last male sperm precedence in this multiple-mated noctuid species when untreated females were sequentially mated with P or F_1 males alternating with unirradiated male moths. Irradiated P males and their F_1 progeny males were nearly as competitive as non-irradiated males, exhibiting 75-79% of the matings and sperm transfer to female at 130 Gy.

The status of inherited sterility in F_1 progeny derived from 130 Gy irradiated males was confirmed by examining the chromosomal aberrations (heterologous translocations) in the primary spermatocytes of F_1 progeny fathered by 130 Gy treated male parents in laboratory

and field cages. Mating assessments of irradiated P males (with 130 Gy) and their F_1 male progeny evaluated at a 5:1:1 sex ratio (Irradiated male: Normal male: Normal female) in laboratory and field simulated cages showed a reasonably effective mating competitiveness (0.69-0.91).

Conclusion: The present study validated the sperm competence and corroborated mating competitiveness of P males irradiated at 130 Gy and their F_1 male progeny in laboratory and field simulated cages, which reflects the effective operational feasibility of this radio-genetic technique for population suppression of *Spodoptera litura*.

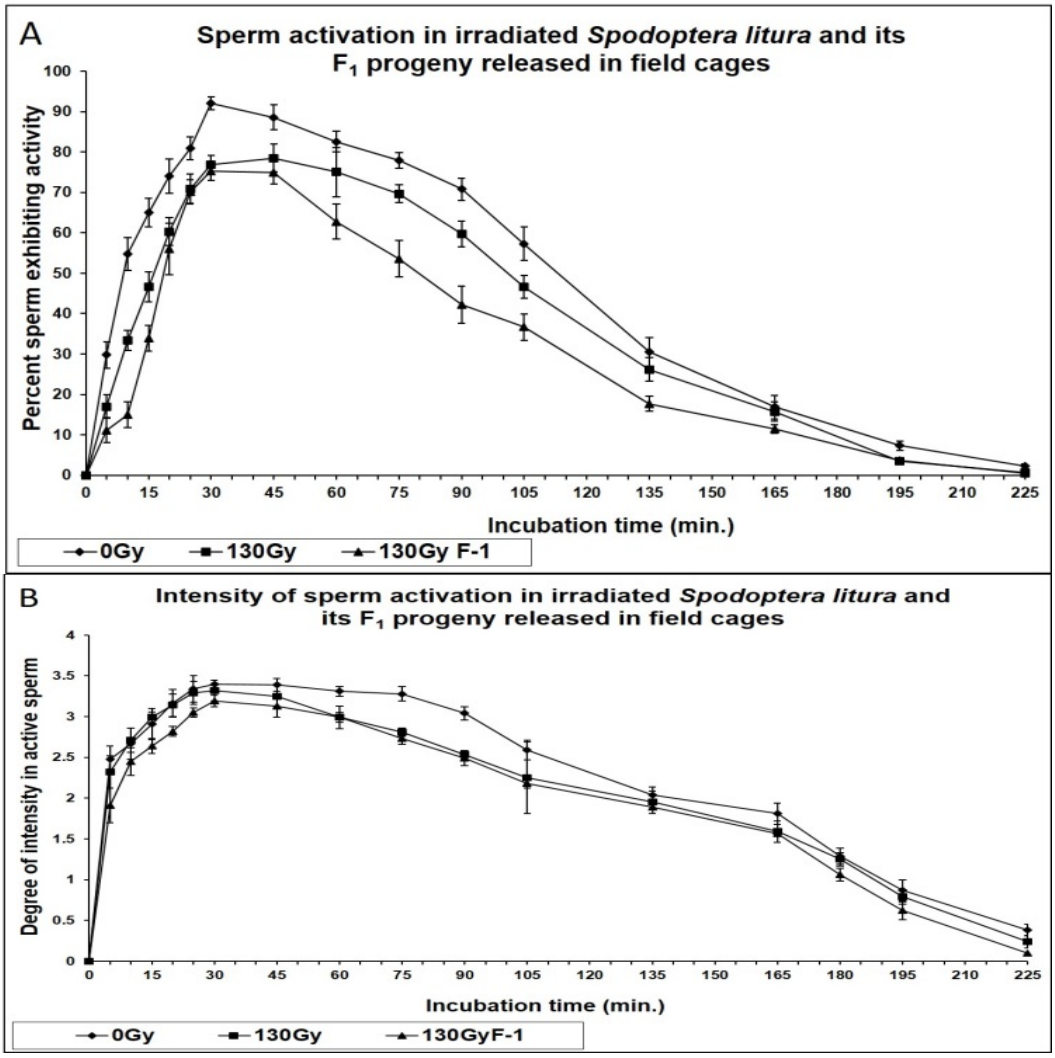


Fig.1*: Sperm activation in irradiated* Spodoptera litura *and its F_1 progeny released in field simulated cages. A: % sperm exhibiting motility, B: Intensity of sperm activation.*

Abstract ID 69

Ascertaining the Efficacy of Gamma Radiation on the Flower Webber, *Maruca vitrata* (Fabr.) (Lepidoptera: Crambidae) to Establish the Inherited Sterility Technique for the Management of this Pigeon Pea Pest in India

Basavaraj PATIL[1], Shivanand HANCHINAL[1], Zubeda KHAN[2], Mahtab ZARIN[2], Rachappa HAVERI[3], Chandrashekhar MALUR[3], Suhas YELSHETTY[3] and Rakesh Kumar SETH[2]

[1]University of Agricultural Sciences, Raichur, Karnataka, India, Email: *bvp2001@rediffmail.com*
[2]Applied Entomology and Radiation Biology Unit, Department of Zoology, University of Delhi, Delhi-110 007, India
[3]Department of Agricultural Entomology, Agricultural Research Station, Kalaburagi-585101, Karnataka, India

Background: The flower webber, *Maruca vitrata* (Fabr.) is becoming a predominant insect pest in all pigeon pea (*Cajanus cajan*) growing areas of southern India. The cryptic behaviour of the larvae, cost, and environmental risks associated with excessive use of chemical insecticides impose serious limitations to manage this pest. Hence, the potential and feasibility of using radiation induced 'Inherited (F_1) Sterility' technique for the management of this pest were studied.

Methodology: Quality mass-rearing of *M. vitrata* was carried out on an optimized semi-synthetic diet. Various reproductive characteristics (viz. mating success, sperm transfer and fertility) of irradiated moths were evaluated up to the F_1 progeny in response to gamma radiation (0-250 Gy). *M. vitrata*'s behavioural and physiological responses vis-a-vis ionizing rays were evaluated in the lab, followed by field studies on mating competitiveness of irradiated insects, sterility and the extent of crop damage, with an aim to identify the gamma dose for operating 'Inherited Sterility' technique.

Results: Radio-sterilization in the Parent generation: The mating success of irradiated parent (P) males crossed with unirradiated females was decreased in comparison to the control (76.3). The longevity of irradiated P males was not affected; however, the oviposition by females crossed with treated P male was reduced at 100-200 Gy. Suppression of reproduction due to crosses of irradiated P males with unirradiated females was determined as 38.6-81.6% at 100-200 Gy, with respect to the control.

Substerilizing irradiation mediated Inherited Sterility: The reproductive behaviour of F_1 adults (derived from irradiated P males) was studied in further crosses. The impact on oviposition was evident in F_1 crosses, with drastic reduction in egg-laying by unirradiated females crossed with F_1 male progeny of 200 Gy treated P males. The fertility of F_2 eggs was significantly reduced with 72-80% reproductive suppression at 150 Gy and complete sterility

in F_1 progeny from 200 Gy irradiated P males. Mating as a crucial parameter in P & F_1 generation was negatively correlated with P irradiation.

The mating success with unirradiated females was > 60% in case of 150 Gy treated P males and their F_1 males, whereas at 200 Gy the mating success of P males with unirradiated females was reduced from 62.5% to 7.5% by their corresponding F_1 males (Figure1).

Radiation induced reduction in longevity of F_1 adults was apparent unlike in the P generation. Mating competitiveness of the released 150 Gy treated P and F_1 males in the lab and field cages was evaluated and correlated with damage, in terms of webbings per plant at different durations after release.

Conclusion: In view of this study, 150 Gy might be considered reasonable to operate F_1 sterility for the suppression of *M. vitrata* populations, whereas studies are in progress on the mating competitiveness of 200 Gy treated P male moths in field cages in order to explore the pragmatic potential of 200 Gy for *Maruca* control through the SIT.

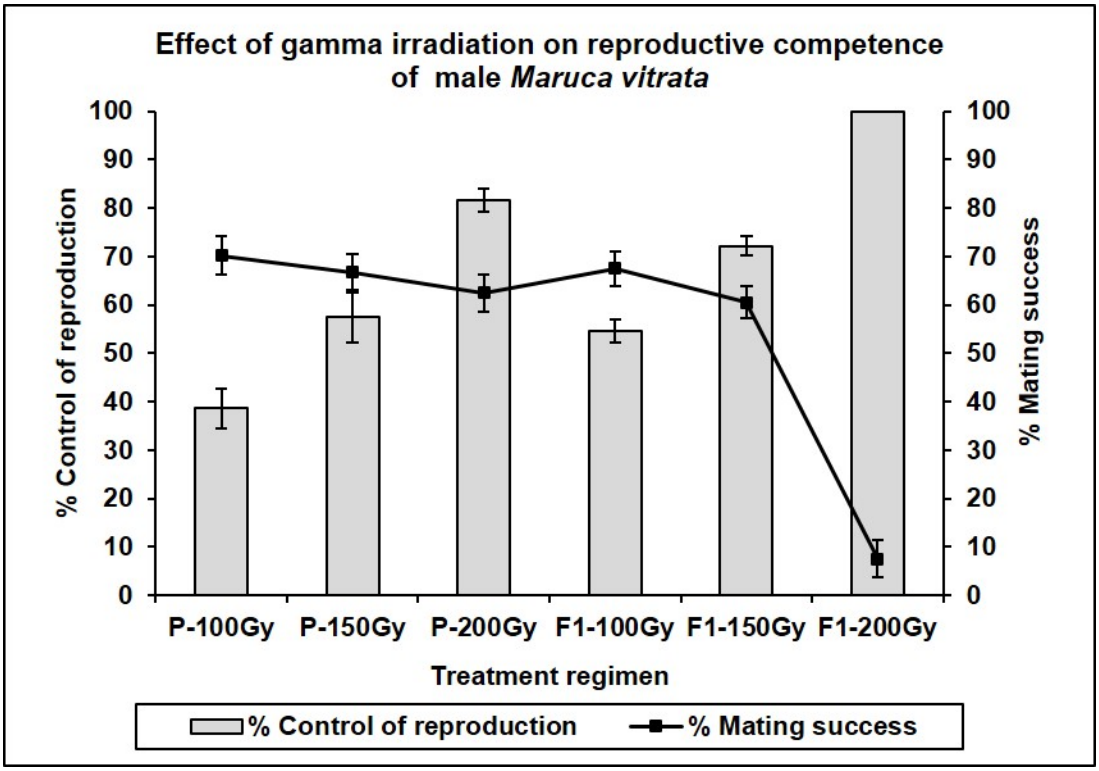


Fig.1. *Effect of gamma irradiation on reproductive competence of* Maruca vitrata *when parent (P) male moths and their F_1 males were crossed with normal female.*

Abstract ID 79

Rearing *Fopius arisanus* (Hymenoptera: Braconidae) on Irradiated Eggs of *Ceratitis capitata*

Beatriz PARANHOS

Embrapa Semiárido, Petrolina, Brazil, Email: *beatriz.paranhos@embrapa.br*

Background: One of the tools to suppress or eradicate the carambola fruit fly, *Bactrocera carambolae*, which is restricted to the northern region of Brazil, is the use of parasitoids. However, the Brazilian native parasitoids do not attack this fruit fly. In this way, *Fopius arisanus*, which attacks eggs and young larvae of the genera *Bactrocera* and *Ceratitis*, was imported. This parasitoid must be multiplied on *C. capitata* to be sent to Amapa State, northern Brazil, which is free of *C. capitata*. Thus, to send parasitized pupae to that place, free of host pest, it would be necessary to irradiate the host before exposing it to parasitism.

Methodology: For this, studies were carried out at Moscamed Facility in Juazeiro-BA, northeastern Brazil, to determine the doses of X-rays that should be applied to egg-hosts, according to their age, in order to obtain higher yields and parasitism rates. The ages tested were 24, 30, 36 and 40 hours of incubation of *C. capitata* eggs, irradiated with doses of 2.5, 7.5, 10 and 12.5 Gy, respectively. For all experiments each repetition was a cage with 10 couples of *F. arisanus*, 10 to 20 days old, with previous experience in parasitism and fed ad libitum with honey and water. The host eggs were offered on soft paper in blocks of agar-water (7%), which were put in the upper screen on the outside of the cage, through which the females could parasitize the eggs, for 24 h.

Results: The irradiation of 24 h old eggs presented negative effects in all evaluated parameters, compared to the non-irradiated ones. After 30 h of incubation, *C. capitata* eggs can be irradiated to avoid pest emergence without negatively affecting egg-pupal yield and parasitism rate by *F. arisanus*. Among the evaluated egg ages, the highest yields were in eggs of 40 h (0.56 ± 0.04) and 30 h (0.55 ± 0.07), higher than those found in eggs of 36 h (0.36 ± 0.04) and 24 h (0.15 ± 0.07) ($F = 10.65$, $P < 0.001$).

The parasitism rate ranged from 58 to 75% between eggs of 24 and 40 h, respectively, but there was no difference between the ages studied ($F = 2.27$, $P < 0.133$). On the other hand, the number of adults obtained was different and superior with eggs of 40 h (95.25 ± 7.06), followed by 36 h (54.42 ± 6.48) and 30 h (51.25 ± 11.26), which were similar and larger than with 24 h eggs (12.45 ± 4.82) ($F = 11.11$, $P < 0.001$). The number of females obtained followed the same pattern as that of adults. It was found that eggs of 40 h generated twice as many females as eggs of 36 and 30 h, and seven times more females than 24 h eggs.

Conclusion: Studies have shown that the best age of *C. capitata* eggs to be irradiated with X-rays and parasitized by *F. arisanus* is 40 h, as it gave the highest pupal yield with a higher female bias.

Abstract ID 81

The Effects of Bacterial Probiotics Fed to Larvae of Queensland Fruit Fly (*Bactrocera tryoni*): Do They Improve Fitness and Performance Under the Sterile Insect Technique?

Lucas SHUTTLEWORTH and Olivia REYNOLDS

New South Wales Government, Department of Primary Industries (NSWDPI), Australia, Email: *olivia.reynolds@dpi.nsw.gov.au*

The Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera, Tephritidae), is native to Australia, and is a pest and biosecurity threat to the $9 billion horticultural industry. *B. tryoni* is controlled using a range of tools including the Sterile Insect Technique (SIT).

SIT involves area-wide, inundative releases of sterile insects to reduce reproduction in a wild population of the same species. Mass-rearing and irradiation of the pupae in SIT programmes reduces fitness and performance of the released sterile insects. Larval gut microbiota may influence health, fitness and behaviour of all phases of the *B. tryoni* life-cycle. It is feasible that domestication, mass-rearing and irradiation may impact the gut microbiota and affect the quality of the mass-reared fruit flies used in SIT programmes.

Using live bacterial species of *Asaia*, *Enterobacter*, *Lactobacillus* and *Leuconostoc* isolated from the midgut of wild larvae as a probiotic supplement, we tested several fitness and performance traits of *B. tryoni*. These included larval development time, pupal weight, time to emergence of adults, sex-ratio, flight ability, survival in field cages and mating competitiveness.

The addition of probiotics to the larval diet could lead to improvements in mass-rearing of *B. tryoni* used in SIT programmes such as, reduced development times, reduced costs of production, and increased competitiveness in the field. 123

Abstract ID 84

Optimising Composition of High Performance Gel-based Larval Diet for Queensland Fruit Fly (*Bactrocera tryoni*) (Diptera: Tephritidae)

Tahereh MOADELI, Bishwo MAINALI, Fleur PONTON and Phillip W. TAYLOR

Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia, Email: *taherehmoadeli@students.mq.edu.au*

Background: The Queensland fruit fly, *Bactrocera tryoni* is Australia's most economically damaging insect pest of horticulture, infesting a vast diversity of commercial crops in eastern states. The sterile insect technique (SIT) is currently being developed as an environmentally-benign and sustainable approach for management of *B. tryoni* through the mass-rearing and release of millions of sterile flies to curtail reproduction of wild populations.

Development of new larval diets with cost-effective ingredients is required for economical mass-rearing of high quality flies for SIT, and this in turn requires detailed information about larval nutritional requirements. The common ingredients of recently developed gel-based larval diet include a protein source (yeast), carbohydrates, lipids and vitamins (wheat germ oil, WGO), preservatives, pH modifiers, gelling agents and water. As a step toward optimization of gel-based larval diets, we assessed performance of *B. tryoni* flies reared on diets with manipulated doses of WGO, and also investigated fatty acids as key components of WGO.

Methodology: *B. tryoni* larvae were reared on two gel-based larval diets that were created by adding 1% of agar to liquid diet formulations. Larval diets were supplemented with 0, 0.03, 0.07, 0.11, 0.15% or 1% WGO. Additionally, to identify key nutritional components of WGO, linoleic, linolenic, oleic, and palmitic fatty acids as well as α -tocopherol (vitamin E) were substituted for WGO individually and in combination. Quality control parameters were evaluated based on standard FAO/IAEA/USDA procedures.

Results: Gel-based larval diets supplemented with WGO outperformed diets without WGO in most QC parameters, especially in terms of pupal number and percentage of fliers. Higher WGO doses of 1.0, 0.15 and 0.11 % produced similarly high levels of performance, but lower doses of 0.07 and 0.03 % produced dose-dependent lower performance.

Diets containing a combination of all tested fatty acids or just unsaturated fatty acids performed as well as diets containing WGO, although addition of vitamin E reduced productivity. Considering individual fatty acids, larval diets containing linolenic acid produced adults with higher percentage of fliers than did larval diets containing palmitic acid, oleic acid or linoleic acid.

Conclusion: Addition of WGO to gel-based larval diets improved percentage of fliers in adult *B. tryoni*. A minimum WGO dose of 0.11% was identified as sufficient to yield full benefits.

Addition of fatty acids to the gel-based larval diet did substitute for WGO, confirming that fatty acids are key to the beneficial effects of WGO. Specifically, the beneficial effects of WGO for production of flight capable adults could be met by incorporating a combination of unsaturated fatty acids or potentially by an alternative, cheaper, source of these key fatty acids.

Key words: Wheat germ oil (WGO), fatty acid, linoleic acid, linolenic acid, oleic acid, palmitic acids, Sterile insect technique (SIT)

Abstract ID 88

Optimization of Semi-synthetic Diet for Quality Mass-rearing of the Legume Pod Borer, *Maruca vitrata* (Fabr.) (Lepidoptera: Crambidae) Towards Employment of 'Inherited Sterility' Technique for Pest Suppression

Rachappa HAVERI[1], Shivanand HANCHINAL[1], Chandrashekhar MALUR[1] Shwetha SURPUR[1], Zubeda KHAN[2], Zarin MAHTAB[2], Basavaraj PATIL[1], Suhas YESHETTY[1] and Rakesh Kumar SETH[2]

[1]Department of Agricultural Entomology, Agricultural Research Station, Kalaburagi, University of Agricultural Sciences, Raichur, Karnataka, India, Email: *rachis1@rediffmail.com*

[2]Applied Entomology and Radiation Biology Unit, Department of Zoology, University of Delhi, Delhi, India

Background: The flower webber, *Maruca vitrata (*Fabr.) is an important tropical pest of grain legumes and has a wide distribution throughout Asia, Africa, South America, and Southern Australia. In recent years, *M. vitrata* is becoming a major insect pest in all pigeon pea growing areas of south India. The hidden orientation of the larvae makes it challenging to manage this pest with insecticides along with the environmental risks associated with their use.

In these circumstances, the legume pod borer management needs to be strengthened through eco-friendly pest management tactics such as the Sterile Insect Technique (SIT) / F1 (Inherited) Sterility technique. To harness this radiogenetic control method, the most pertinent pre-requisite is to establish a standardized, cost-effective mass-multiplication methodology to obtain mass-culture of quality (viable) insects that would exhibit a good growth index, proper reproductive competence and maximum biotic potential.

Methodology: In this context, the evaluation of four semi-synthetic diets: (i) D-OOD diet (Ochieng et al. 1981), (ii) D-OO diet (Onyango and Ochieng-Odero 1993), (iii) D-NEW diet (Wang et al. 2013), (iv) Modified D-OOD diet, was carried out to study their potential to support various biocharacteristics (growth, development and reproductive competence) of *M. vitrata*, and compared with the potential of natural food (pigeon pea), for a series of generations, in order to have diet refinement and its optimization for mass-rearing of insects.

Results : Comparison of developmental and adult parameters of six successive generations of *M. vitrata* reared on the above mentioned diets revealed that the Modified D-OOD diet supported maximum egg hatch, and substantial survival up to ultimate instar larva (L5) and pupa, at 85.3%, 79.8% and 73.0%, respectively. This data indicated that the Modified D-OOD diet was superior over the other diets (D-OOD, D-New and D-OO), while it was at par with the natural host.

Maximum oviposition was also evident on the Modified D-OOD diet that was comparable with insects reared on the pigeon pea (host). Furthermore, the egg incubation period, total larval duration and pupal period did not vary significantly on the Modified diet and the natural host. Similarly, the per cent egg hatch and mating success did not differ significantly in both cases. Further, the moths reared on the Modified D-OOD diet could retain a reasonably effective reproductive competence after irradiation with sterilizing / partial sterilizing doses.

Conclusion: Among different diets compared, the Modified D-OOD diet was superior to other diets in terms of growth index, ovipositional capacity, and reproductive potential of this legume pod borer. This comparative study on the rearing potential of different diets clearly indicated that the Modified D-OOD appeared to be suitable for mass-rearing of viable insects depicting good growth index and higher biotic potential. The reproductive performance of irradiated moths reared on Modified D-OOD diet confirmed the efficacy of this diet and indicated its use for mass-rearing of viable moths needed to employ the SIT/F1 sterility technique.

Abstract ID 99

Ceratitis capitata Control on Table Grapes in Spain by the Mass-trapping System Cera Trap®

Nuria SIERRAS, Cándido MARÍN, Anna BOTTA and Ricard BROSSA

Bioibérica, Barcelona, Spain, Email: *nsierras@bioiberica.com*

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is a worldwide agricultural pest with an extremely high invasive potential, which makes this species particularly difficult to control effectively. Pesticide treatments have historically been used against this insect through aerial and terrestrial treatments. However, lately there is a public demand for more benign alternatives techniques due to adverse effects of broad-spectrum insecticides on humans, non-target organisms and the environment. Additional concerns exist because of the potential development of insecticide resistance.

Mass-trapping techniques are currently one of the most common control methods applied against Mediterranean fruit fly as an alternative to standard insecticide sprays. Taking into account these considerations, Cera Trap® was developed as a pesticide-free attractant formulation of natural origin, based on a liquid protein mixture obtained by an exclusive method of enzymatic hydrolysis. Cera Trap has a strong attraction capacity for Mediterranean fruit fly and with higher ratios of young females.

Many field trials were carried out worldwide over the last ten years showing that Cera Trap is an effective system against Mediterranean fruit fly. Cera Trap is registered in Spain as plant protection product under Regulation 1107/2009 on the following crops: citrus, seed and stone fruits and tropical and subtropical fruits, and field trials have been carried out last year to extend its use to table grapes.

Field trials carried out in Murcia (Spain) on three different varieties of table grapes (*Vitis vinifera* cv. Itum, Autumn Royal and Crimson) are described comparing the effectiveness of Cera Trap against a standard attract-and-kill system. Results suggest that Cera Trap decrease fruit fly infestation and fruit damage providing a good opportunity for IPM strategies.

Abstract ID 101

Intrinsic and Synthetic Stable Isotopes for Reliable Identification of Wild and Mass-reared Queensland Fruit Flies in SIT Programmes

Polychronis REMPOULAKIS, Phillip Warren TAYLOR, Bishwo MAINALI and Anita ANDREW

Macquarie University, Sydney, Australia, Email: *polychronis.rempoulakis@mq.edu.au*

Background: Stable isotopes are naturally occurring, non-radioactive forms of elements that have similar number of protons and different number of neutrons. They have extensive use in ecology for investigation of trophic levels and in physiology for the study of metabolic processes. Stable isotopes are also suitable for use in applied entomology to mark and identify insects.

The highly polyphagous Queensland fruit fly (*Bactrocera tryoni*) is a significant threat for Australian horticulture, causing extensive damage to a wide range of commercial and non-commercial crops. Environmentally benign management practises are increasingly important for controlling *B. tryoni* populations. Among those methods, the Sterile Insect Technique (SIT) is gaining ground as key component of Area Wide Integrated Pest Management (AW-IPM). Reliable identification of released flies is a pre-requisite for successful application of SIT.

Methodology: In this study we assessed the feasibility of using stable isotope signatures as markers to differentiate between mass-reared and wild *B. tryoni* flies. As an additional marking method, we also investigated the potential of larval diet enrichment using 13C D-Glucose for the permanent marking of mass-reared *B. tryoni* flies. With the use of an elemental analyser and isotope ratio mass-spectrometry we measured the δ13C and δ15N values of wild flies collected from numerous locations in Australia and in New Caledonia, and laboratory flies originating from two mass-rearing facilities. All natural hosts of *B. tryoni* fly are C3 plants with a δ13C isotopic signature of ca. −28‰ VPDB, whereas most laboratory larval diets contain sugar from sugarcane, a C4 plant with δ13C isotopic signature of ca. −11‰ VPDB.

Additionally, for the laboratory flies we compared isotopic signatures of flies originating from two larval diets, one based on lucerne chaff and containing sugar from C4 plants and one based on carrot without added sugar, and we followed the changes in δ13C values of adults as they fed on diets similar to or different from their larval diets. For the enrichment experiments we used a gel diet especially designed for *B. tryoni* fly larval rearing, and added small amounts of 13C D-Glucose to reach a value of δ13C ca.+50 ‰ VPDB . We analysed isotopic composition of larvae, pupae, and adults.

Results: Our findings indicate that mass-reared *B. tryoni* flies (with δ 13C ‰ values ranging from ~-23 to ~-15 ‰) can be reliably distinguished from wild *B. tryoni* flies (δ 13C ‰ -29) regardless of their geographical origin. We also found that the δ13C values measured in different body parts of adults reflect the adult diet, although clear differences between mass-reared and wild flies persist. Finally, a detailed study of the intake of elevated levels of 13C in larval diet during all metabolic stages showed that it is possible to mark released flies with this isotope.

Conclusion: Stable carbon isotopes offer an effective and economical approach for reliable identification of sterile flies in situations where captured flies cannot be reliably identified because of inadequate marking with fluorescent dyes.

Abstract ID 107

Effects of Gamma Irradiation on the Digestive Enzymatic Activities of Mediterranean Fruit Fly *Ceratitis capitata* (Diptera: Tephritidae)

Haitham HAMDEN [1], Meriem M'SAAD GUERFALI [1], Ferjani DHAOUADI [1] and Ameur CHERIF [2]

[1]National Center for Nuclear Sciences and Technologies (CNSTN), Tunis, Tunisia, Email: *heithem_hamden@yahoo.fr*
[2]LBBGRV, Tunisia

Background: For SIT, the reduction of pest population size is achieved through mass-releases of reproductively sterile male insects into a wild-type population. It has been shown that the sterilizing irradiation procedure affects negatively the performance of sterile Mediterranean fruit fly, *Ceratitis capitata*, males in the environment by damaging midgut tissue and/or disrupting the disbiotic conditions and intestinal microbiota of their alimentary tract.

Considering the improved performance of sterile males after probiotic enrichment of their adult or larvae diet and the negative effects of irradiation on the activity of digestives enzymes, the overall purpose of this study was to characterize the effects of irradiation on the carbohydrase and proteases activity of *Ceratitis capitata* and to determine how supplementation of the larval diet with a probiotic bacteria can alleviate the negative effect of irradiation on the activity of Mediterranean fruit fly digestive enzymes.

Methodology: The probiotic bacteria were added to the standard Mediterranean fruit fly mass-rearing larval diet at 109 CFU/ml concentrations. Two days before emergence, pupae were exposed to gamma radiation at a dose of 110 Gy. The dissection of the digestive system of 50 flies was carried out in a drop of NaCl (0.15M). The guts homogenates obtained were used for the enzyme assay. The activity of carbohydrase enzymes was determined by estimation of p-nitrophenol released used as substrates: PNPαglu, PNPβglu, PNPαgal and PNPβgal. The amount of liberated nitrophenol was estimated by measuring absorbance at 405 nm. Activities toward disaccharides (sucrose and maltose) and polysaccharides (starch and inulin) were determined by the Bernfeld method. The azocaseinase activity was estimated using a solution of azocasein 1.5% as substrate. The soluble peptides were measured by absorbance at 440nm. The homoglobinase activity was estimated using a solution of hemoglobin 1% as substrate. The soluble peptides were measured by absorbance at 750 nm.

Results: The enrichment of larval diet by probiotic bacteria enhanced the activity of most of the studied carbohydrases and proteolytic enzymes after emergence for both irradiated and non-irradiated samples. We noticed an improved αgal activity after enrichment (5.73±0.73) for the non-irradiated samples compared to the control (4.7±0.37). Although irradiation reduces the αgal activity, sterile males reared in the probiotic enriched larvae diet had significant more αgal activity (3.9±0.1) compared to the sterile control males (2.4±0.1).

For the proteolytic activity, the azocaseinase activity pre- (15.26±0.63) and post-irradiation (12.43±0.52) was improved compared to the control (6.46±0.55 and 0.9±0.71, respectively). Likewise, the probiotic enrichment significantly improved the hemoglobinase activity pre- (17.5±0.37) and post-irradiation (14.5±0.64) compared to the control (6.43±0.83 and 4.46±0.23, respectively).

Conclusion: The Enterobacter cloacae enrichment of larval diet enhances the activity of most carbohydrases and proteolytic enzymes studied after emergence for both the irradiated and the non-irradiated samples.

Abstract ID 108

Probiotic Potential of *Ceratitis capitata* Gut Microbiota for the Enhancement of Sterile Insect Technique

Haitham HAMDEN [1], Meriem M'SAAD GUERFALI [1], Salma FADHEL [1], Wafa MARZOUKI [1] and Ameur CHERIF [2]

[1]National Center for Nuclear Sciences and Technologies (CNSTN), Tunis, Tunisia, Email -Tunisia, Email: *heithem_hamden@yahoo.fr*
[2]LBBGRV

Background: The concept of exploitation of the intestinal microbiota for production of competitive *Ceratitis capitata* sterile males for SIT application was developed by a microbial adjunct in the insect diet. The evaluation of the probiotic potential of the bacterial strain must consider the biology of the insect, the mass-rearing conditions and the irradiation process. The adoption of logical criteria for the *in vitro* selection can result in strains that are capable of performing effectively in the gastrointestinal Mediterranean fruit fly tract. The selected probiotic should survive in the larval diet, persist in the insect gut, resist to the irradiation, improve the intestinal microbial balance and improve the biological and reproductive parameters of sterile insects.

The present study aimed to evaluate the probiotic potential of intestinal bacteria strains isolated from the wild adult Mediterranean fruit fly gut. These strains were evaluated for eligible probiotic traits adapted to SIT protocols such as tolerance to irradiation, biofilm formation and EPS production. Additionally, the isolated strains were tested *in vivo* to study their effects on some quality and reproductive parameters of produced insects. The candidate probiotic strain that fulfils the established criteria could therefore be used as a novel probiotic strain.

Methodology: One hundred colonies were aseptically isolated from intestinal tracts of wild *C. capitata* using LB agar medium. These colonies were characterized using 16S-rRNA gene sequencing analysis. To evaluate their radio sensitivity, each isolated colony was exposed to a gamma radiation dose of 110 Gy in a cobalt-60 source and viable cells were counted and expressed in CFU/ml. The ability of each colony to colonize and to persist in the gut of the insect was evaluated by biofilm formation (using the crystal violet method) and EPS production (using the phenol-sulfuric acid method). Finally, the isolated colonies were exploited as probiotic in larval diet and the collected pupae was used to determine the pupal weight, flight ability and survival.

Result: Blast analysis of the 16S rRNA gene sequence identified 84 isolates as *Enterobacter cloacae*, 4 as *Lactococcus lactis*, 3 as *Morganella morganii*, 3 as *Pluralibacter gergoviae*, 3 as *Enterobacter asburiae*, 1 as *Providencia rettgerii*, 1 as *Klebsiella pneumoniae* and 1 as *Rhanella aquatilis*.

The results also show that *E. cloacae* has the best probiotic potential by resisting to gamma radiation and by recording the best performance in biofilm formation (1.951±0.048) and EPS production (309.59µg/ml±11.51) compared to the other bacteria and the control. The pupae collected after larval diet enrichment with *E. cloacae* presented also the highest pupal weight (8.6±0.028), the best percentage of flight ability (92.83±0.28) and survival rate (91.11±2.22).

Conclusion: *Enterobacter cloacae* can be considered as an interesting candidate to be used as probiotic in the mass-rearing process of the Mediterranean fruit fly for SIT purposes.

Abstract ID 110

Diversity of the Bacterial Community of the Reproductive System of the Mediterranean Fruit Fly, *Ceratitis capitata* (Diptera: Tephritidae) and the Trapping Activity of Metabolites from the Cultivable Species

Haitham HAMDEN[1], Meriem M'SAAD GUERFALI[1], Kamel CHARAABI[1], Wafa DJOBBI[2] and Ameur CHERIF[2]

[1]National Center for Nuclear Sciences and Technologies (CNSTN), Tunis, Tunisia, Email: *heithem_hamden@yahoo.fr*
[2]LBBGRV

Background: Insects harbour diverse microorganisms in their intestinal tract and other tissues. These microbes and their hosts have developed complex interactions that range from pathogenesis to obligate mutualism. The intestinal tract of adult *Ceratitis capitata* harbours a diverse bacterial community composed mostly of members of the Enterobacteriaceae family. This bacterial community affects several key features for the successful application of the Sterile Insect Technique (SIT) such as male longevity, sexual performance, carbon metabolism and fixation nitrogen. These findings have stimulated research on the isolation and use of these bacteria as probiotics to be added in adult or larvae diet during the mass-rearing of the Mediterranean fruit fly.

In this study, our primary goal was to identify the bacteria inhabiting the reproductive system of the male and female mass-reared *Ceratitis capitata* VIENNA 8 strain, then to determine the effect of irradiation on this community and to evaluate the chemotaxis of *C. capitata* to the metabolites produced by this bacteria.

Methodology: One hundred bacterial colonies were aseptically isolated from the reproductive system of wild males, wild females, wild females mated with wild males, VIENNA-8 males and VIENNA-8 irradiated males *C. capitata* using LB agar medium. These colonies were characterized based on 16S-rRNA gene sequencing analysis using universal bacterial primer 5'-S-D-Bact-0008-a-S-20-3' and 5'-S-D-Bact-1495-a-S-20-3'. After fermentation, 1 ml of each isolated bacteria culture was transferred to a small cotton ball, which was placed, into a fruit fly trapping apparatus. Two hundred medflies (100 females and 100 males) were released into the cage. After 24h, the number and gender of the trapped flies was recorded.

Results: The PCR 16S-rRNA analyses revealed that the Enterobacteriaceae family is the dominant bacterial group in the reproductive system of *C. capitata*. This profile undergoes some changes after irradiation treatment of the VIENNA 8 mass-reared strain, which is expressed by the appearance of some pathogenic strains such as *Pseudomonas* sp.

Our bioassays demonstrated that the metabolites produced by *Klebsiella oxytoca* trapped a greater number of *C. capitata* adults than the control ($F=9.26$; $df1=6$; $df2=14$; $p<0.05$). However, the metabolites produced by *K. oxytoca*, *E. aerogenes* and *E. cloacae* were more

attractive to *C. capitata* adult males and *E. aerogenes*, *K. pneumonia* and *K. oxytoca* were more attractive to females.

Conclusion: In this study, Enterobacteriacea constituted the dominant population in the reproductive system of *C. capitata*. Irradiation stimulates the appearance of pathogenic bacteria such as *Pseudomonas* sp. Finally, we found that *Klebsiella oxytoca* and *Enterobacter aerogenes* metabolites trapped the greatest number of female and male *C. capitata* adults.

Abstract ID 115

Genomic Engineering of Fruit Fly Pest Species to Improve their Control by Biologically-based Methods

Alfred HANDLER and Jianwei LI

USDA, Agricultural Research Service, Gainesville, Florida, USA, Email: *al.handler@ars.usda.gov*

The goal of this research is to improve the efficiency and safety of the sterile insect technique (SIT) by developing new genetically modified strains of fruit fly pests that give rise to sterile male-only progeny. Our approach was to create a temperature-sensitive mutant allele, tra-2-ts, for the transformer-2 (tra-2) sex-determination gene in *Drosophila suzukii*, which is necessary for female sex-specific differentiation in *D. melanogaster* and tephritid fruit flies. This was achieved by using CRISPR/Cas9 gene-editing to replace the wild type Dstra-2 sequence with an in vitro mutagenized sequence known to encode tra-2-ts in *D. melanogaster*.

Based on *D. melanogaster* studies, tra-2-ts chromosomal females (XX;AA) should develop as normal fertile females at the permissive temperature of 16-18°C, but at a non-permissive temperature of 29°C, they are expected to develop as phenotypic males that are sterile. While tra-2 does not have a somatic effect in XY males, it does have a germ-line function, and therefore XY;tra-2-ts males should develop normally at the permissive and non-permissive temperatures, but should be sterile at 29°C. Thus, a tra-2-ts strain should breed normally at 16°C, but when progeny are reared from embryos at 29°C, it is expected that all zygotes will develop as phenotypic sterile males. The gene-edited sequence also included an IE1-DsRed marker gene 3' to the Dstra-2-ts sequence, allowing the identification of mutant flies by DsRed fluorescence, that were inbred at 16°C to create a homozygous strain.

Progeny were then reared at 29°C to assess the mutant phenotype, but unexpectedly there was no survival at this temperature past the pupal stage, with further tests showing that temperatures above 26°C were lethal for *D. suzukii*, though flies reared at <26°C could survive at 29°C as adults. Nevertheless, at 26°C XY;Dstra-2-ts males developed normally but were sterile, while XX;Dstra-2-ts individuals developed as phenotypic intersexuals with a bias to male morphology. This included fully developed foreleg sex combs, and male-like posterior segmental pigmentation and genitalia. Most notably, similar to the *D. melanogaster* tra-2-ts strain, female-specific yolk protein gene transcription could be turned on at 16°C and turned off at 29°C in XX;Dstra-2-ts flies.

Although the full phenotypic effect of the Dstra-2-ts mutation at 29° could not be assessed in *D. suzukii*, this study does provide proof of principle for creating the mutation by CRISPR/Cas9 gene-editing, resulting in a female to male sexual reversion at non-permissive temperature. Thus, it is expected that similar genetic modifications will have the potential for

creating new highly efficient, cost effective and environmentally safe strains for improved SIT in several pest insect species.

Abstract ID 119

Pilot Studies on the Optimization of the SIT through Gamma Radiation for the Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

Venkata Rami Reddy POLURU[1], Ashok B. HADAPAD[2], Soumya BEVANAMAR[3], S. SHASHANK[3], Ramesh S. HIRE[4] and Kamala Jayanthi D. PAGADALA[3]

[1]Division of Entomology and Nematology ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake P. O., Bengaluru 560089 India, Email: *pvreddy2011@gmail.com*
[2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India
[3]ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake P. O., Bengaluru 560089 India
[4]Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

The Sterile Insect Technique (SIT), though a widely tested and established component of area-wide management of fruit flies in several countries, is yet to make a mark in India. The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), polyphagous in nature, is one of the economically important pests of mango and is also of quarantine importance. The export of Indian mangoes to the USA, Europe and Japan is linked to fruit fly free produce. Currently, male annihilation technique (MAT) using methyl-eugenol traps in combination with other pre- and post-harvest treatments is being widely adapted to manage fruit flies in mango. Lack of awareness about traps among growers as well as unwillingness of a section of them to adapt the technology has made the MAT-based IPM falling short of desired level of fruit fly control.

Considering the lack of information on the feasibility of SIT for area-wide management of *B. dorsalis* in India, a pilot project was initiated in 2013 at ICAR-Indian Institute of Horticultural Research, Bengaluru in collaboration with the Bhabha Atomic Research Centre (BARC), Mumbai, India. Standardization of the optimum dose of gamma radiation to induce male sterility, mating behaviour of irradiated males and an artificial diet for mass-production of fruit flies are the objectives of the project.

Initially the culture of *B. dorsalis* was maintained on banana and guava fruits till the artificial diet was developed. The pupae, five to six day old, were exposed to different doses of cobalt-60 γ radiation ranging from 30-100 Gy and the adult emergence was recorded. It was observed that 50 Gy was optimum, which resulted in highest adult emergence with sterilised males. *B. dorsalis* adult emergence varied from 12.53-80.47 per cent and survived from 35-56 days in different doses of gamma irradiation. There was complete failure of the establishment of F_1 generation resulting from mating of irradiated male flies with wild females. The feasibility and scope for large-scale implementation of SIT for management of *B. dorsalis* in India are discussed.

Key words: Bactrocera dorsalis, mango, SIT, India, male sterility, AW-IPM

Abstract ID 124

Distribution and Molecular Characterization of Endosymbiotic *Wolbachia* Bacteria Associated with Different Indian *Bactrocera* Fruit Fly Species

Ashok B. HADAPAD[1], Ramesh S. HIRE[1] and Chandra S. PRABHAKAR[2]

[1]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay- 400 085, Mumbai, India, Email: *ahadapad@barc.gov.in*
[2]Bhabha Atomic Reserach Centre/Bihar Agricultural University, Sabour, India

Background: The fruit flies of the genus *Bactrocera* are major pests of vegetables and fruit crops in India and damage around 30-100% of produce. The damaged fruits and vegetables are unsuitable for human consumption, commercial use and trade. The Sterile insect technique (SIT) has been implemented successfully to control several insect pests including fruit flies. Endosymbiotic bacteria like *Wolbachia* induce cytoplasmic incompatibility (CI) in arthropods. Recently, attempts were made to explore CI as a method to suppress natural populations of arthropod pests and to integrate it with the SIT. This method could be a potent strategy to control insect pests in area-wide insect management programmes. In the present study, five *Bactrocera* fruit fly populations were collected from different agro-climatic regions of India and characterized for prevalence and distribution of *Wolbachia* in fruit flies.

Methodology: Different *Bactrocera* species samples were collected from 11 agro-climatic conditions of India using methyl-eugenol and cue-lure pheromone traps during the years 2012-2016. The fruit flies belong to the melon fly (*B. cucurbitae*), Oriental fruit fly (*B. dorsalis*), guava fruit fly (*B. correcta*), peach fruit fly (*B. zonata*) and pumpkin fruit fly (*B. tau*). The total DNA was extracted from individual fruit flies and the quality of DNA was ensured using 12S rRNA gene amplification. The DNA samples showing positive amplification were further screened for *Wolbachia*-specific gene (Wspec), *Wolbachia*-specific protein (wsp) gene and MLST genes. The positive amplicons were sequenced and phylogenetic relationships of *Wolbachia* in Indian fruit fly species were studied.

Results: 657 samples of 5 different *Bactrocera* species were screened for *Wolbachia* infection using *Wolbachia* specific 16S rRNA gene and sequenced. Around 324 fruit fly samples were positive for *Wolbachia* infection and an average of 46.83% of *Wolbachia* prevalence was observed among the *Bactrocera* species sampled. Prevalence of *Wolbachia* was differed with respect to species and geographic locations. *Bactrocera tau, B. dorsalis* and *B. cucurbitae* showed 67, 57, and 52% of *Wolbachia* prevalence, respectively, while, lower infection levels were observed in *B. correcta* and *B. zonata*.

Based on the Neighbour-Joining (NJ) analysis, three major *Wolbachia* clades were formed. The sequences under study showed strong clustering with Supergroup-A of *Wolbachia* which is aparasitic in nature. High frequencies of melon fly samples were found to be positive for gatB followed by coxA in MLST analysis. Thus, the *Wolbachia* screened in this study could

be used to induce cytoplasmic incompatibility and could be used as biocontrol agent for the management of important agricultural insect pests and vectors.

Conclusions: Prevalence of endosymbiotic bacteria like *Wolbachia* was observed in 5 Indian *Bactrocera* sp. sampled. The prevalence of *Wolbachia* was studied in each fruit fly species, location and belongs to Supergroup-A. Understanding the *Wolbachia* distribution in Indian fruit flies will facilitate development of eco-friendly control measures to control these *Bactrocera* species.

Abstract ID 128

From BioLure to Magnet MED: Over 20 years of Suterra's Commitment to the Sustainable Control of the Mediterranean Fruit Fly

Daniel CASADO, Ignacio DE ALFONSO and Cristina ALFARO

Suterra Europe Biocontrol SL, Valencia, Spain, Email: *dani.casado@suterra.com*

The Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) is a pest of a wide range of fruit crops, and it has a great economic importance world-wide due both to crop losses and export restrictions. The monitoring of this pest underwent a revolution in the mid-1990s. Classically, *C. capitata* had been monitored with trimedlure (a powerful male attractant but with no effect on females) or protein-based aqueous baits (unspecific, cumbersome and impractical). In 1995 and 1997 successive studies demonstrated that a synthetic blend of ammonium acetate, putrescine and trimethylamine constituted a powerful attractant for both sexes of *C. capitata*.

Suterra immediately started the commercialization of lures of this patent-protected blend of attractants that soon became, and still is, the standard for attraction of *C. capitata*. This has allowed the implementation of reliable industrial lures, first used for monitoring, but later also for direct control by mass-trapping. To implement mass-trapping, a high rate of fly traps (typically 50–75 per hectare) are deployed, baited with these lures and loaded with an insecticide-impregnated strip. Other companies have developed their own alternative lures which always include ammonium acetate, trimethylamine hydrochloride and a short-chain diamine.

Throughout the years, Suterra's lures have undergone several changes. Originally, each of the three components of the blend was formulated independently in a separate lure (lures commercialized under the trademark BioLure). This approach was known as Tripak. Later on, to simplify handling, the three lures were integrated into a single unit with three compartments (or pouches), one per component of the blend, and were commercialized as Unipak BioLure. Recently, Suterra has released a new version of the product with a single pouch, where all three components are combined into this single pouch. Despite the changes performed to the product over time to simplify its use, the attractant blend has been kept untouched without detriment to performance.

Tripak, later Unipak and other similar lures have been long used for mass-trapping, becoming a wide-spread and sustainable Mediterranean fruit fly management, ideal for IPM programmes. Despite its efficacy, mass-trapping can result cumbersome. The traps, the insecticide and the lures are typically provided separately, requiring considerable labour to assemble them. Furthermore, fly traps are bulky what makes transportation and logistics difficult and inefficient.

To solve these limitations Suterra has developed Magnet MED, a ready-to-use attract-and-kill product. The patented design consists of an envelope coated with insecticide and that contains a Unipak BioLure. The flies are attracted to the device, walk on its surface and drop dead to the ground. Magnet MED is easier and faster to deploy than any mass-trapping system, reduces the need of product manipulation by the operators, improves dramatically the efficiency of transportation and logistics, and provides at least the same level of control. Magnet MED is currently registered in 10 countries and used on more than 20,000 ha.

Abstract ID 136

Expression Pattern of the Mediterranean Fruit Fly-specific Odorant-binding-protein CcapOBP83a-2 in Response to Gamma-irradiation

Kamel CHARAABI, Meriem M'SAAD GUERFALI, Heithem HEMDEN, Salma FADHEL and Mouldi SAIDI

LR Biotechnology and Nuclear Technologies, National Center for Nuclear Sciences and Technology (CNSTN), Tunisia, E-mail: *msaad_tn@yahoo.fr*

Background: The population suppression success of Mediterranean fruit fly, *Ceratitis capitata* (Wied) integrating the sterile insect technique (SIT) depends mainly upon the ability of sterilized males to locate and mate with wild females. Irradiated males are known to be at a mating disadvantage in terms of attractiveness to wild females, courtship behaviour, or acceptance of the courtship by females. Considerable progress has been made in understanding Mediterranean fruit fly mating behaviour, and it is known that odorant binding proteins (OBPs) and odorant receptors play important roles in this process. Despite the evident importance of OBPs in Mediterranean fruit fly behaviour, no studies have been performed on the effects of gamma-irradiation on OBPs expression of sterilized medflies. Here we analysed the expression pattern of the OBP CcapOBP83a-2 gene throughout development and it's response to different doses of gamma-irradiation, by quantitative reverse transcription PCR.

Methodology: Total RNA was extracted from antennae (150 pairs) of one day-old (1d) immature, four day-old (4d) sexually mature virgin and four day-old (4M) mated individual male flies of non-irradiated and irradiated samples using Trizol (Invitrogen, CA), according to the manufacturer's instructions. After DNAse treatment (DNA-free, TaKaRa, Kyoto, Japan), RNA integrity was determined by formaldehyde agarose gel electrophoresis and quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE, USA). For each sample 200 ng of the extracted total RNA was transcribed into cDNA using the iScriptTM cDNA Synthesis Kit (Biorad) and the ABI 7500 real-time PCR System were used for CcapOBP83a-2 gene real-time PCR. Negative controls without template were included in each experiment. Two Mediterranean fruit fly reference genes (GAPDH2 and G6PDH) were used for normalization. Relative gene expression data was estimated using the $2^{-\Delta\Delta CT}$ method.

Result: Real-time quantitative PCR analyses confirmed a trend of increasing transcript abundance is evident as a consequence of male maturation for CcapOBP83a-2. Olfactory behaviour of most insects displays remarkable phase changes associated with different physiological status, such as sexually immature and mated adults. However, in response to gamma irradiation, the olfactory CcapOBP83a-2 gene displayed a slight, but significant, reduction in transcript abundance. The results indicated that treatment with different

sterilizing doses of gamma irradiation resulted in decreased transcription of the CcapOBP83a-2 gene at different developmental stages (one day-old (1d) immature, four day-old (4d) sexually mature virgin and four day-old (4M) mated individual male flies), compared to the untreated samples. Real-time quantitative PCR revealed that CcapOBP83a-2 gene expression was slightly decreased during the gamma radiation treatments and reached a peak in the dose of 145 Gy.

Conclusion: The OBP CcapOBP83a-2 gene displayed a slight reduction in transcript abundance in the antenna of the Mediterranean fruit fly, in response to gamma irradiation. This can be responsible for the inferiority of sterile males compared with wild males.

Abstract ID 137

Effects of Gamma Radiation on the Sperm Viability of Mediterranean fruit Fly *Ceratitis capitata* (Diptera: Tephritidae)

Salma FADHEL, Meriem M'SAAD GUERFALI, Wafa MARZOUKI, Lotfi SILLINI and Mouldi SAIDI

LR Biotechnology and Nuclear Technologies, National Center for Nuclear Sciences and Technology (CNSTN), Tunisia, Email: *msaad_tn@yahoo.fr*

Background: The Sterile Insect Technique (SIT) is a biological, environmentally-friendly method of pest suppression or elimination that fits well into the area-wide approach of preventive insect pest management. This technique has become the main component of the strategy for the area-wide suppression or elimination of *Ceratitis capitata* (Wiedemann) populations.

Gamma radiation damages genetic material, and the resulting chromosome breaks induce dominant lethal mutations in reproductive cells. The treated males will compete for mates in the wild to transfer their sterile sperm. Sperm viability is one of male adaptations to post-copulatory sperm competition in insects and the proportion of live sperm in a male's semen determines paternity success. A sperm viability test is a common assay of semen quality, providing new advances in ecology and evolution.

In this study, our purpose was to determine the temporal dynamics of sperm viability in mass-reared *C. capitata* males irradiated in the late pupal stage with doses ranging from 0 to 145 Gy.

Methodology: The *C. capitata* flies were obtained from a colony of the VIENNA 8 genetic sexing strain (GSS) produced in the Tunisian Mediterranean fruit fly rearing facility in the National Center of Nuclear Sciences and Technology (CNSTN). Virgin males were anesthetized with nitrogen and dissected immediately. We removed the testes from sexually mature males and placed them in phosphate-buffered saline (PBS). Sperm were released from the tissue and diluted with PBS. Equal volumes of sperm and live/dead stain (LIVE/DEAD viability/cytotoxicity assay kit, L-7011) were mixed in an Eppendorf vial and incubated in the dark at room temperature for 45 minutes. Stained sperm was viewed with a standard flourescein excitation optical filter at 20X or 40X, depending on the size of sperm. We examined 500 sperm from each individual of fly (fewer if numbers were limited) and recorded whether each sperm had a red head (dead) or was entirely green (live). Data were analysed with Statgraphics one-way Anova and Tukey HSD.

Results: Sperm viability level was significantly affected by radiation doses (P=0.000). The fertilization ability of irradiated sperm would be inferior to that of non-irradiated sperm. Our study demonstrated that the number of viable sperm in irradiated males of *C. capitata* was

significantly affected by radiation (145 Gy) compared with untreated samples respectively (81.1 % (±1.37) and 99.8% (±0.07)).

Conclusion: We used a simple assay for recording the viability level of sperm in male's testes of *C. capitata* mass-reared Vienna 8 strain. The preliminary results show a decrease in the sperm viability level compared to the control. Irradiation could directly and/or indirectly affect fertilization success through effects on sperm viability.

Abstract ID 152

Isolation and Characterization of Some *Bacillus thuringiensis* Strains from Tunisian Soil with Insecticidal Activity against Mediterranean Fruit Fly: *Ceratitis capitata*

Yassine MABROUK[1], Nihed MAJDOUB[2] and Omrane BELHADJ[2]

[1]National Center for Nuclear Sciences and Technologies (CNSTN), Tunis, Tunisia, Email: *yassine.mabrouk@gmail.com*
[2]Faculty of Science of Tunis, Tunisia

Background: Chemical insecticides have been used for several decades and have shown harmful effects on the environment. In addition to this, these chemicals are non-specific and are implicated as carcinogens. Consequently, some researchers have focused their works on developing alternative methods to synthetic chemicals for controlling pests. Among these alternatives are the use of microorganisms as biological control agents, such as *Bacillus thuringiensis*. The purpose of this research was to study the effects of toxins of *B. thuringiensis* against Mediterranean fly *Ceratitis capitata*.

Methodology: The screening of larvicidal activity of *B. thuringiensis* strains was performed using 4 different concentrations of spore crystal mixture (100, 75, 50 and 25 ppm) alongside one control. In each case, 10 *C. capitata* larvae were exposed in triplicates. The strain of *B. thuringiensis* S40, which showed the highest larvicidal activity, was used for the extraction of crystal proteins. Proteins extracted from the Bt-S40 pellet were further purified by ammonium sulfate precipitation, and the 40% fraction was then subjected to DEAEA and–sephadex A-50 ion exchange chromatography, followed by gel filtration chromatography on sephadex G-100.

Results: Among 70 isolates, 15 showed significant larvicidal activity. Five isolates of *B. thuringiensis* are distinguished by a larvicidal effect which is greater than ≥ 90% on *C. capitata*. The difference of the larvicidal activities observed in the isolates studied can be explained by the variability of the isolates proven by the analysis of the protein profiles on polyacrylamide gel under denaturing conditions. The concentrations of the spore crystal mixture, which kill 50% of the exposed populations in standard bioassays (LC50), were observed between 100 ppm and 50 ppm. The highest larvicidal activity was noticed with Bt-S40 strain. Proteins purification steps showed 5 major protein peaks. The third peak protein fraction was active against *C. capitata* larvae with an estimated LC50 = 30 ng per larvae.

Conclusion: The preliminary results of this study encourage us towards the characterization of the insecticidal activity produced by *B. thuringiensis* strains for use in biological control of *C. capitata.*

Key words: Bacillus thuringiensis, larvicidal activity, endotoxins, *Ceratitis capitata*

Abstract ID 154

Influence of Adult Diet in the Mating Performance and Longevity of *Bactrocera correcta* in Viet Nam

Hien NGUYEN THI THANH, Trang VU THI THUY, Thanh VU VAN, Lien HA THI KIM and Khanh LE DUC

Plant Protection Research Institute Hanoi, Vietnam, Email: *thanhhien1456@gmail.com*

Background: The guava fruit fly, *Bactrocera correcta* Bezzi, is one of the serious pests attacking many kinds of fruits in Viet Nam. In order to improve the effectiveness of SIT application for the control of this fly, studies were carried out in which the effect of protein in the adult diet on sexual maturity and longevity of *B. correcta* was evaluated.

Methodology: *B. correcta* flies used in this study originated from Binh Thuan province and had been cultured for 8 generations on a sweet potato larval diet at the Entomology Division of the Plant Protection Research Institute (PPRI), Viet Nam. The flies were maintained in the laboratory at 26 ± 2°C, 60-70% RH and a photoperiod of 10:14.

Effect of protein in the adult diet on sexual maturity: Thirty pairs of newly emerged *B. correcta* flies were released into cages (30 cm x 30cm x 40 cm) covered by cloth mesh. Water was supplied and refreshed every 2 days. The flies were provided with three different diet treatments, which were (i) sugar, (ii) hydrolyzed yeast; and (iii) a mix of hydrolysed yeast: sugar (1:4 proportion). Every afternoon (17:00 - 22:00 h), the percentage of matings was observed until flies reached the age of 20 days. Mating pairs were removed from the cages and recorded. They were not returned to their cages after the end of mating. The procedure was repeated three times for each treatment.

Longevity of sterilized males and females on protein and sugar diets: Each pair of newly emerged sterilized fly was released in cylindrical cage (30 cm height and 8 cm radius) and supplied with only water by a wet sponge placed on the top of cylindrical cages. The adult diets were studied at three different treatments, which were (i) sugar, (ii) hydrolysed yeast; (iii) a mix of hydrolysed yeast: sugar (1:4 proportion). 100 fly couples per treatment were tested. Daily observations and collection of the data on dead adults (male/ female) were performed until all flies were dead. Dead flies were removed during daily checks.

Results: The initial results showed that the first age of sexual maturation of laboratory flies was 7-days old and 8-days old when fed on a mixed diet of hydrolyzed yeast: sugar and only on a sugar diet, respectively. The flies only feeding on hydrolysed yeast diet were dead after 4 days of emergence. The highest percentage of mating was 7.19% for 11-day-old of flies fed on sugar and 40.8% for 10-day-old of flies fed on hydrolysed yeast: sugar (ratio 1:3).

The longevity of sterilized males was 30.7 days, 28.1 days and 3.2 days feeding on the mixture of hydrolyzed yeast: sugar (ratio 1:3), only sugar and only hydrolysed yeast, respectively. Likewise, this period for females was 32.5 days, 30.5 days, and 3.7 days.

Abstract ID 159

Determination of *Ceratitis capitata* (Mediterranean Fruit Fly) Feed Sources by Microbial Community Analysis

Harald BERGER[1] and Rebecca HOOD-NOWOTNY[2]

[1]Biocide Berger, Vienna, Austria, Email: *office@biocide-berger.at*
[2]Austrian Institute of Technology, Tulln, Austria

Ceratitis capitata, the Mediterranean fruit fly, is spread worldwide from the Mediterranean area to America and Asia, causing extensive damage to a wide range of fruit crops. Area-wide pest management efforts including sterile insect technology (SIT) to suppress and eradicate the fruit fly were successfully applied in California, Chile and Mexico, but the threat of reintroduction of the pest remains. Therefore monitoring fruit fly distribution and quarantine efforts remains crucial in prohibiting further spread.

C. capitata, being very polyphagous, is able to feed on many different fruits. This has to be taken into account when considering area-wide pest management efforts. If the fly is able to hide in biological niches and wild hosts away from the main crops that are treated during the management efforts, it will easily migrate back to these crops once the efforts have ceased, voiding the efforts.

We want to present tools that allow monitoring the feed sources and thereby the biological niches from which trapped flies have come from. *C. capitata* was reared on 4 different feed sources and total DNA extraction was performed on single flies. A 16S ribosomal gene fragment was amplified to prepare a library of all bacterial strains associated with the flies. These amplicon libraries have been sequenced by MiSeq high throughput sequencing technology to obtain a quantitative representation of the microbial community associated with each fly.

Using Random Forest classification, specific microbial community compositions could be associated with specific feed sources. These results were further compared to results obtained by stable isotope technology on the same experimental setup.

Abstract ID 161

Efficiency Enhancement of Sterile Insect Technique by Vectoring Entomopathogenic Fungi

Ahmed Mohammed ALJABR[1], Abid HUSSAIN[1], Muhammad RIZWAN-UL-HAQ[1] and Hassan AL-AYEDH[2]

[1]King Faisal University, Hofuf, Saudi Arabia, Email: *aljabr@kfu.edu.sa*
[2]King Abdulaziz City for Science and Technology, Saudi Arabia

Background: Red palm weevil is a severe pest of a wide range of genera of palms with a worldwide distribution. *Rhynchophorus ferrugineus* control is primarily based on the frequent application of synthetic pesticides. However, environmental pollution, possible deleterious effects on non-target animals, applicator safety issues, concerns over human health and the development of resistance among red palm weevils to insecticides have forced scientists to search for and develop new and sustainable methods of biological control including entomopathogenic fungi and the sterile insect technique (SIT).

Results: Our laboratory SIT results showed that the standardized dose of gamma radiation (15 Gy) tremendously affect not only the oviposition potential of *R. ferrugineus*, but also the hatchability of eggs.

On the other hand, laboratory pathogenicity bioassays using insect pathogenic fungi specially *Metarhizium anisopliae* and *Beauveria bassiana* against *R. ferrugineus* adults impart significant mortality allowing the integration with SIT to achieve the full potential of these techniques. Our results suggested that sterile red palm weevil adults infected with fungal conidia transmit disease that along with reduced oviposition and hatchability enhanced the mortality of red palm weevils.

Conclusion: The vectoring of *Metarhizium anisopliae* and *Beauveria bassiana* conidial suspensions to gamma radiated sterile red palm weevil adults could be promising red palm weevil-controlling technology.

Abstract ID 163

New Molecular Tool for Improving SIT in *Ceratitis capitata*: Evaluation of Sperm Transfer During Mating by Absolute Quantification RT-PCR

Elena LLACER-ARCHELOS[1], Marta CATALA[1], Alberto URBANEJA[1] and Meritxell PÉREZ-HEDO[2]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain, Email: *ellacer@ivia.es*
[2]Universitat Jaume I (UJI), Castellón de la Plana, Spain

Background: Currently the assessment of male sexual behaviour to determine quality of sterile mass-reared *Ceratitis capitata* (Wiedemann) flies inside Sterile Insect Techniche (SIT) programmes, relies on a required periodic quality control field cage test that mainly takes into consideration the ability of sterile male flies to compete with wild males for mating with wild females, but additional procedures as duration of mating or dissection of females to count sperm heads by microscope, are also included to estimated sperm transfer. However these last procedures are tedious and costly both in time and in labour. Therefore, a more accurate and faster procedure to quantify sperm transfer would help to improve male sexual quality assessment. This study was carried out to establish a new procedure that fulfils that purpose.

Methodology: To assess the quantity of sperm transferred by *C. capitata* males to females during mating, we adapted an absolute quantification RT-PCR method to be applied under the required conditions. The standard curve used to run in parallel with unknown samples was obtained by cloning a target sequence of male DNA and afterwards preparing a series of known dilutions of this standard material. The male DNA material was extracted from spermathecae after mating for almost 120 minutes and was multiplied by conventional PCR. Different primers and temperatures were tested to select those that showed the most visible results to be used in the RT-PCR procedure. The developed method was applied to quantify sperm transfer of males of 3 ages (4, 11 and 18 days after adult emergence) which were allowed to mate during different periods of time (10, 60 or 120 minutes) with 4 days old females. In order to validate this molecular method, part of the samples were used to determine number of sperm by the conventional microscope sperm heads counting.

Results: The standard curve was satisfactorily set up and its efficiency fit acceptable values in all of the tests. In the mating experiment, differences in sperm transfer were detected. For quantified samples, sperm transfer was higher in the first days of male sexual maturity for the same length of mating. For the same age of males, the longer the mating lasted, the more quantity of sperm was transferred.

Conclusion: This method is able to quantify sperm transfer from males to females during mating and could be applied in periodic quality control test of sterile mass-reared adults. Furthermore, it is a useful tool to improve the knowledge of mating in *C. capitata* and could

be applied to know how different factors applied to Mediterranean fruit fly males, such as diet ingredients or treatments enhancing male performance, may affect sperm transfer.

Abstract ID 173

Influence of Mating Status on the Pathogenicity of *Providencia rettgeri* against a Mass-reared Strain of the Mediterranean Fruit Fly *Ceratitis capitata* (Diptera: Tephritidae)

Meriem M'SAAD GUERFALI[1], Wafa DJOBBI[1], Salma FADHEL[1], Heitham HAMDEN[1], Wafa MARZOUKI[1] and Claude CHEVRIER[2]

[1]National Center for Nuclear Sciences and Technologies (CNSTN), Sidi Thabet, Tunisia, Email: *msaad_tn@yahoo.fr*
[2]Institut de Recherche sur la Biologie de'l Insecte (IRBI), Tours, France

Background: Mass-reared sterile males are clearly inferior to wild males in both survival and sexual competitiveness. Apparently, the reasons for this low competitiveness stem from their colonization, and the irradiation, handling and release processes. Many studies suggested that the microbiota associated with the fly's digestive tract may play a significant role in their fitness during the various stages of the host's life cycle.

Providencia species exhibit pathogenicity against some Diptera, so we can assume that the relatively high infection rates of up to 22% at 5 days of age of irradiated Mediterranean fruit fly males could affect their fitness. It has been suggested that mating may have immunosuppressive effects in females diptera to allow the limited resources to be shunted from immunological requirements to reproductive needs. Moreover, mating can cause decreased survival after infection with a pathogen in females, but post-mating immunosuppression may not be universal. Therefore, we investigated the effect of mating status of infected mated males and females by quantifying their mortality and bacterial load at different post-infection times, and also by quantifying the relative density of cDNA amplicons of cecropin gene expression.

Methodology: Mating procedure: Male and female flies were collected as virgins three days post-emergence. They were allowed to copulate and males were removed from the females shortly after the end of copulation to prevent additional courting or copulation attempts. Flies were infected by *P. rettgeri* through injection 2.5 h after mating.

Bacterial load assay: Bacterial load of flies infected with *P. rettgeri* was assessed. The number of colony forming units (CFU) was recorded using a system allowing the number of viable Enterobacteriacae to be calculated based on the number and position of colonies.

RNA extraction and cecropin relative density: Total RNA from infected virgin and mated males and females was extracted and reverse transcribed to cDNA. The primer design was developed for cecropin gene isoform 2 (X70029) as follows: 5'-GCTGTCATCTTTGCCGTTTT-3' (forward primer) and 5'-GCAACGCTGAAGGGATAAC-3' (reverse primer). PCRs were standardized using G6PDH

specific primers: 5'-CCGACGAGCAGGCAAAATATG-3' (forward primer) and 5'AGACGGACGCGGGTAAGG-3' (reverse primer).

Results: Our results showed that after mating, mortality in female Mediterranean fruit fly was reduced. Significant levels of cecropin mRNA were detected within an hour after bacterial injection reaching a peak a few hours later. It was 4 times higher than with control virgin females. On the other hand, we observed that mating protected females against infection by *P. rettgeri* by triggering an immune response with the secretion of doubled levels of cecropin compared to infected virgins reducing the virulence of the bacteria.

Conclusion: It seems that there is a kind of protection conferred to the mated females through the bactericidal activity to protect the stored sperm after mating as was the case for the andropin gene with *Drosophila* non-infected males. However, generally the mating effect seems to be pathogen-dependent due to microbial heterogeneity and not host dependent.

Abstract ID 175

Probiotic Enrichment and Consequences on Hsp 70 and Cecropin Expression of Mass-reared Strain of *Ceratitis capitata* (Diptera: Tephritidae)

Meriem M'SAAD GUERFALI[1], Kamel CHARAABI[1], Heithem HAMDEN[1], Salma FADHEL[1], Wafa MARZOUKI[1] and Ameur CHERIF[2]

[1]National Center for Nuclear Sciences and Technologies (CNSTN), Sidi Thabet, Tunisia, Email: *msaad_tn@yahoo.fr*
[2]Higher Institute of Biotechnology, Tunisia

Background: The composition of intestinal microbiota is of prime importance for the fitness of sterile insects. Rearing stressors could impair this composition as it is the case with the increase in the level of some pathogens such as *Pseudomonas* sp. Enrichment with probiotics of the larval diet or at the adult stage is recommended in insectaries. In recent studies probiotics could repair the imbalance due to mass-rearing stressors and contribute to the insect intestinal wall integrity, acting against pathogens and supporting immune system growth. In the present work we wanted to characterize the endogenous role of the probiotic enrichment by providing some information on the resistance mechanisms to environmental stress and ecological adaptation. The work focused on the quantification of a biochemical stressor indicator expressing the Hsp 70 and an indicator of immune system function for the Mediterranean fruit fly, the cecropin.

Methodology: Probiotic administration: The strain *Enterobacter cloacae* with high probiotic potential was isolated from the digestive tract of wild caught medflies and added to the standard larval diet. Irradiation process: Collected pupae of the Mediterranean fruit fly VIENNA 8 strain were irradiated at 90 Gy two days before emergence. RNA Extraction and First Strand cDNA Synthesis: Total RNA of 1 day and 5 day flies was extracted and reverse transcribed to cDNA. Real-time PCR: Single coding sequences of the gene HSP 70 and cecropin were amplified. The endogenous control was run using G6PD and G2PDH. Statistical analysis: ΔΔCT values were calculated using fold differences of 2-ΔΔCT. The means and standard deviations were calculated from experiments performed in triplicate and are presented as the n-fold differences in expression.

Results: In our study the expression of the two genes was not significantly induced by irradiation, while it was accentuated after probiotic enrichment in the first day of emergence. The gene expression levels were not significantly different between runs (ANOVA, P=0.45).

Conclusion: The administration of probiotic bacteria had a significant effect on the up-regulation of stress and immune-related genes, while the mass-rearing process and irradiation impaired these traits. The application of probiotic bacteria is an excellent mode of

immunostimulation and also proves that enhanced production of AMPs could be a possible mode of action of probiotic bacteria.

Abstract ID 176

Molecular Regulation of Pupal Diapause Development and Termination in Chinese Citrus Fly *Bactrocera minax*

Yong-Cheng DONG, Zhen-Zhong CHEN and Chang-Ying NIU

College of Plant Science & Technology, Huazhong Agricultural University, Hubei Sheng, China, Email: *ccdyc@163.com*

Background: The Chinese citrus fly, *Bactrocera minax*, is an economically important pest of citrus. It is univoltine and overwinters by means of pupal diapause lasting for six months. The long pupal diapause is a barrier for basic and applied research for this target pest.

Methodology: To better understand the pupal diapause in this pest, we investigated: 1) the influence of environmental temperature and 20-hydroxyecdysone (20E) on its diapause development and termination; 2) the differentially expressed genes and proteins in diapausing individuals during diapause development, as well as in diapause-terminated individuals by 20E-treatment using high-throughput RNA-seq and iTRAQ technologies based on our previously constructed genetic information of the *B. minax* transcriptome.

Results: The results showed that pupal diapause development and termination in *B. minax* is strongly influenced by chilling conditions. Increasing cold exposure led to significantly and consistently faster adult emergence and improved synchronization of adult emergence.

By topical application, 20E can effectively terminate the pupal diapause in a dose-dependent manner and significantly shorten pupal duration to reach adult emergence. 20E response genes, including ecr, broad and foxo, were up-regulated within 72 h, indicating that these genes are involved in pupal metamorphosis and diapause termination processes.

Four digital gene expression libraries including early-, late-, post-diapause and diapause-terminated individuals were constructed and sequenced. In total, 4,355 unigenes showing the differences expressed across four libraries revealed major shifts in cellular functions of cell proliferation, protein processing and export, metabolism and stress response in pupal diapause.

When diapause was terminated by 20E, many genes involved in ribosome and metabolism were differentially expressed, which may mediate diapause transition. The gene sets involved in protein and energy metabolisms varied throughout early-, late- and post-diapause, while in proteomic analysis, 1,659 differentially expressed proteins were enriched in lipid and amino acids metabolism, signalling transduction. These genes might be associated with signal transmitting and utilization of reserves, switching the diapause development to direct development. A combined analysis of transcriptomic and proteomic data showed signal transduction pathways were significantly involved in diapause transition.

Conclusion: This knowledge facilitates the rearing of *B. minax* by manipulating pupal diapause termination, and provides extensive genetic resources on pupal diapause development, which sheds new light on the possible mechanisms involved in pupal diapause in this pest.

Key words: dormancy, developmental arrest, energy storage, seasonality

This study was funded by International Atomic Energy Agency (via Research Contract CRP No. 17153 and CRP No. 18269) and the Joint National Science Foundation of China-Israel Science Foundation project (No. 31661143045).

Abstract ID 180

Identification and Manipulation of Symbiotic Bacteria Associated with *Bactrocera dorsalis* and *Bactrocera minax*

Awawing ANDONGMA, Yulei WANG, Adnan MOHAMAD, Kanjana KHAESO, Akami MAZARIN and Chang-Ying NIU

[1]College of Plant Science & Technology, Huazhong Agricultural University, Wuhan, China, Email: *niuchangying88@163.com*

Background: Insects are associated with bacteria that affect their fitness and survival in diverse ways. These symbionts play vital roles in insect development and reproduction. In recent years, the intricate relationship between fruit flies and their symbiotic bacteria is gaining more global attention as a strategy to control pests. In this study we used *Bactrocera dorsalis* and *Bactrocera minax* as model insects to understand bacterial community structure, effects and transmission patterns, which could pave the way for the development of efficient and sustainable pest management programmes.

Methodology: Culture-dependent and pyrosequencing techniques were used to identify the spectrum of bacteria associated with *B. dorsalis* and *B. minax*. In addition, the possible roles of isolated bacteria and their transmission patterns were studied.

Results: In *B. minax* the Proteobacteria were dominant in all life stages. It was realized that egg disinfection reduced hatching rates, but the addition of antibiotics to rearing diets had no effect on the number of copulations, longevity and fecundity. On the other hand, adding the bacteria *Enterobacter cloacae*, *Pantoea agglomerans* and *Enterococcus faecalis* significantly increased fecundity. Similarly the addition of *Klebsiella oxytoca* or *Enterococcus faecalis* to diets significantly increased the flies' life span, but this significant increase is not observed when flies were previously fed with antibiotics. Feeding flies with sugar diet had no effects on insect longevity; however, the addition of *Klebsiella oxytoca* to sugar diets significantly increased copulation and fecundity.

Regarding *B. dorsalis*, there was a shift in the dominant bacteria group from Proteobacteria in the developmental stages to Firmicutes in the adults. Continuous feeding with antibiotics significantly reduced adult fecundity and longevity. However the addition of different bacteria did not have any effects on the measured fitness parameters except *Enterococcus phoeniculicolas* that shortened the pupal developmental time and increased their survival ratio.

After bacteria transformation with fluorescent proteins, *Enterobacter cloacae* and *Enterococcus faecalis* formed bacterial films in *Bactrocera dorsalis* gut 48 hours after ingestion. In addition, *Enterococcus faecalis* administered to the eggs were found in the adult gut indicating the possibility of vertical transmission.

Conclusion: Symbiotic bacteria associated with these flies play a vital role in their survival, which could be exploited in their management. In addition, the relation between different fruit flies and their symbionts is complex and closely depends on host of other factors.

Key words: Symbiotic bacteria, Bactrocera dorsalis, Bactrocera minax

This study was funded by International Atomic Energy Agency (via Research Contract CRP No. 17153) and the Joint National Science Foundation of China-Israel Science Foundation project (No. 31661143045).

Abstract ID 184

Evaluation and Transfer of Quality Control Procedures from Lab to Industrial Scales for the *Bactrocera dorsalis* Salaya1 Genetic Sexing Strain

Nidchaya AKETARAWONG, Sujinda THANAPHUM, Kamolthip LAOHAKIEAT and Siriwan ISASAWIN

Mahidol University, Nakhon Pathom, Thailand, Email: *nidchaya.akt@mahidol.ac.th*

Background: *Bactrocera dorsalis* is one of the most destructive fruit fly pests worldwide, causing fruit yield loss and international trade limitation. The Sterile Insect Technique (SIT) is an ecologically-friendly and species-specific insect birth control method that is applied as a component of Area-Wide Integrated Pest Management. To maximize the effectiveness of the SIT, the use of sterile male-only strains (or genetic sexing strains, GSS) is required. A GSS for *B. dorsalis* named Salaya1 was developed based on brown-white pupal colour dimorphisms and characterized. At the system prototype level, it has been evidenced to be genetically stable and mates competitively with wild populations. Recently, the *Regional R&D Training Center for Insect Biotechnology* (RCIB) has been started up with a new mass-rearing facility (ca. 700 m^2) for basic and translational research as well as technology transfer activities related to the effective use of the GSSs at an AW-IPM-SIT scale. RCIB is being formally operated in 2017. In its fully operational capacity, it can produce up to 3 million sterile male-only files. Therefore, the development of filter-rearing and standard mass-rearing practices for the management of the facility and technology transfer are very essential.

Methods: Data of routine quality control tests have been recorded and compared between the bisexual (Phayathai1) and genetic sexing strains (Salaya1) of *B. dorsalis* at laboratory scale. Pupal eyes colour changes related to developmental stages will be recorded and photographed. In addition, adult hatching rate and sex ratio have been collected. Only the sex reversal rate of Salaya1 strain was recorded. The experiments have been done over one year or 10-12 generations. Quality control procedures have been transferred from the laboratory to industrial scale at the mass-rearing facility (RCIB). The same parameters will be compared between Salaya1 strain at the prototype and industrial scales. Again, the experiments will be done over a significant period of time, (i.e., one year or 10-12 generations).

Results: At the laboratory scale, the egg hatch rate (%) varied between bisexual (79.3±3.8; Phayathai1) and GSS (47.1±13.0; Salaya1) strains. Pupal recovery rate (%) was similar (Phayathai1: 87.3±12.8, and Salaya1: 72.9±16.9). Adult emergence (%) varied between the bisexual (80.4±5.7) and GSS (67.3±10.7) strains. Additionally, productivity rate (%)varied as expected between the two different strains: 55.5±6.8 (Phayathai1) and 22.6±6.7 (Salaya1).

Conclusions: Relying on preliminary data of two to five generations, several parameters of quality control of Salaya1 were following theoretical expectations when comparing the

bisexual and data of published GSS strains. However, the experiments will be done over the significant period to confirm these results.

Abstract ID 190

The Complete Mitochondrial Genome of *Bactrocera tsuneonis* (Diptera: Tephritidae) by Next-generation Sequencing

Yue ZHANG, Shiqian FENG, Lijun LIU, Zihua ZHAO and Zhihong LI

China Agricultural University, Beijing Shi, China, Email: *zhangyuejacky@yeah.net*

Background: Bactrocera tsuneonis, generally known as Japanese orange fly, is considered to be a major pest of commercial citrus crops. It has a limited distribution in southern China and Japan, while it has the potential to invade areas outside of Asia. More genetic information on *B. tsuneonis* should be shared in order to facilitate the development of effective measures for its prevention and control in the world.

Mitochondrial genomes are widely used in evolutionary and phylogenetic studies in different insect groups due to their rapid mutation rate and short coalescence time. Up to now, mitochondrial genomic information has been reported for only 18 species of Tephritids, not including *B. tsuneonis*.

Methodology: The identified adult sample of *B. tsuneonis* was collected in Sichuan province of China and preserved in 100% ethanol. The genomic DNA was extracted using the DNeasy DNA Extraction kit (QIAGEN). The prepared library was sequenced on an Illumina hiseq 2500. Then de novo assembly was performed on the next generation sequencing data by using Geneious 10.0. We picked up the mitochondrial sequence with the “map to reference” strategy where partially known mitochondrial fragment sequences (from cox1 gene) were used for alignments with all sequences, and then we got the target sequence of about 16 kb. Protein-coding genes and two rRNA genes were identified by BLAST searches in NCBI and then confirmed by alignment with homologous genes from closely related species. The tRNA genes were identified using the tRNAscan-SE and MITOS WebServer. The mitochondrial genome sequences of *B. tsuneonis* and other species of Tephritidae which were released in NCBI were used for phylogenetic analysis.

Results: The mitochondrial genome sequences of *B. tsuneonis* was a typical circular molecule consisting of 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes and a non-coding region (A +T-rich control region). The structure and organization of the molecule were typical and similar compared to the published homologous sequences of other fruit flies in the Tephritidae. The phylogenetic tree presented a close genetic relationship between *B. tsuneonis* and *B. minax*.

Conclusion: The whole mitochondrial genome of *B. tsuneonis* can be used in further studies of evolutionary biology, prevention and control.

Key words: Bactrocera tsuneonis, *mitochondrial genome, next-generation sequencing, phylogenetic analysis*

Abstract ID 191

Comparison of Bacterial Communities Associated with *Bactrocera correcta* (Diptera: Tephritidae) from Four Geographical Populations

Lijun LIU, Yang DAI and Zhihong LI

China Agricultural University, Beijing Shi, China, Email: *ljliu@cau.edu.cn*

Background: The guava fruit fly, *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae), is a destructive insect pest of a wide range of fruits and vegetables. This pest is an invasive species and is currently distributed only in Yunnan province of China. Previous studies have shown that associated bacteria play an important role in the development and reproduction of their hosted fruit flies. In order to investigate the function of associated bacteria, the bacteria communities associated with *B. correcta* should be systemically studied.

Methodology: In this study, we researched the bacterial diversity of *B. correcta* from four wild populations, including two Chinese populations (Jinghong and Ruili) and two Thailand populations (Bangkok and Chiangmai), using DNA-based approaches. The associated bacteria were identified via the analysis of 16S rRNA gene libraries at the 3% distance level.

Results: The identification of 10 operational taxonomic units (OTU) of bacteria associated with Jinghong population was affiliated with 4 classes and 10 genera (*Citrobacter*, *Enterobacter*, *Providencia*, *Orbus*, *Desulfovibrio*, *Lactococcus*, *Vagococcus*, *Enterococcus*, *Staphylococcus* and *Wautersiella*). Four OTUs were assigned to 1 class and 1 genus (*Enterobacter*) for the Ruili population.

The bacteria of Bangkok population had 10 OTUs, which can be attributed to 2 classes and 6 genera (*Citrobacter*, *Enterobacter*, *Providencia*, *Orbus*, *Lactococcus* and *Pseudomonas*). While for Chiangmai population, 1 class (7 OTUs) was grouped into 4 genera (*Enterobacter*, *Citrobacter*, *Klebsiella* and *Kluyvera*).

Enterobacter was the most preponderant bacterial taxa for all Ruili (100%), Bangkok (45.8%) and Chiangmai (76.2%) populations, while *Orbus* was the most preponderant bacteria for Jinghong population, with the percentage of 26.2%. The bacterial diversity of Jinghong population is higher than that of the other three populations.

Conclusion: There was no difference of the bacterial diversity between Chinese ad Thailand populations. This study for the first time provides a systemic comparison of the symbiotic bacteria of *B. correcta* from different geographical populations.

Key words: Bactrocera correcta, *bacteria diversity, wild population, geographical populations*

Abstract ID 193

The Sterile Insect Technique (SIT) as a Novel Approach to Study Polydnaviruses of Parasitic Wasps in their Hosts

Christa SCHAFELLNER and Georg MICH

BOKU-University of Natural Resources and Life Sciences, Vienna, Austria, Email: *christa.schafellner@boku.ac.at*

Background: Polydnaviruses are endosymbiotic viruses that play an essential role in parasite-host interactions involving parasitic wasps and their lepidopteran hosts. Thousands of wasps in the braconid and ichneumonid subfamilies are each associated with a specific braco- or ichnovirus. The virus-carrying wasps parasitize a large diversity of lepidopteran hosts, including important pests in agriculture and forestry. Viral particles are produced in the wasp ovaries and injected into the host larvae together with wasp eggs. In the host body, the viral DNA integrates into the lepidopteran host cell DNA. Viral genes are expressed and interfere with the host‘s immune responses, ensuring wasp larvae survival inside the host body.

We used two closely-related wasps, the gregarious species *Glyptapanteles liparidis* and the solitary *Glyptapanteles porthetriae*, and their associated bracoviruses, GlBV and GpBV, to investigate the specificity of the immunosuppressive function in their common host, larvae of the gypsy moth, *Lymantria dispar*, a serious forest defoliator in Europe, Asia and North America.

The biochemical and physiological interactions between hosts, parasitic wasps and polydnaviruses are quite complex and difficult to study. Radiation can be used to modify or terminate certain parasitoid processes that affect host physiology and development, e.g., by sterilizing the wasps.

Methodology: To separate the wasp egg from its associated virus, we exposed female wasps to γ-radiation from a cobalt-60 source that is normally used in SIT programmes. Treatment doses were adjusted to 96 Gy to achieve temporary sterilization, i.e. within a time frame of 48 hrs the irradiated wasps injected 100% non-viable eggs, but intact virus particles (pseudoparasitism). The pseudoparasitized hosts were developmentally delayed, displayed morphological abnormalities and high mortality during pupation or in the pupal and adult stage. The irradiated wasps exhibited reduced oviposition rates and a low total number of eggs laid per female, but the wasps regained partial fertility and their longevity was not affected. The pseudoparasitized *L. dispar* host larva containing virus particles from one wasp was then implanted with fertile, virus-free eggs from the other wasp. This procedure was done reciprocally with both wasp species. We dissected the hosts at regular intervals and recorded the fate of the implanted wasp eggs.

Results: Virus particles of *G. liparidis* supported the development of *G. porthetriae* parasitoids, but the wasp larvae did not emerge. The reverse experiment produced almost the

same result. Similarly, the combination of both virus particles and eggs of *G. porthetriae* resulted in fully developed parasitoids which did not emerge. Successful emergence of parasitoids from the host larva was observed only when both virus and eggs of *G. liparidis* were combined.

Conclusion: The results from the cross-protection experiments show that the virus of one wasp species can protect the egg of the other species from encapsulation, but does not promote complete development of the wasps indicating a high level of specificity in these wasp-virus associations.

Abstract ID 194

Defence against Insect Pests Using Peaceful Applications of Nuclear Technology

Basim ALMAYYAHI

KUFA University, Kufa, Iraq, Email: *basimnajaf@yahoo.com*

Background: Nuclear techniques are used as part of the integrated approach to control insect pest that destroy crops and spread disease. Disease and pest destroy more than third of crop before and after harvesting.

Methodology: Insect pests can be controlled using the sterile insect technique (SIT- nuclear technology). The SIT, an environmentally friendly alternative to insecticides, involves the laboratory mass-rearing of the target insects, which are then sterilized with gamma radiation. They are released into the wild and mate with the target females, with no offspring produced.

Results: Over time, insect populations shrink and are eventually fully suppressed, reducing the need for pesticides. For example, the SIT was used to eradicate the Mediterranean fruit fly, a threat to 250 species of fruit and vegetables, from Chile and Mexico, and from parts of Guatemala and the USA. By suppressing insect pest populations with SIT, pesticide use world-wide can be reduced.

Conclusion: The technique has improved incomes for thousands of producers. The pesticide-free agricultural products grown in developing countries satisfy international food safety requirements, and thereby can increase the access of rural agricultural communities to valuable export markets. The use of the SIT has reduced the fruit fly populations, which helped double earnings from the agricultural export of tomatoes, bell peppers and papaya, while providing badly needed new jobs.

Abstract ID 198

Advanced Integrated Pest Management - Chemical Control Via Microencapsulation

Borjana LUBURA and Miguel GIMENO-SIERRA

Jesmond BioScience GesmbH, Vienna, Austria, Email: *bl@jesmond.com*

Background: The further development of pest management strategies coupled with specific actives increases the success rate of insect pest suppression or eradication programmes. One of the novel insecticides represent microencapsulated sprayable formulations (CS = capsule suspensions) which belong to environmentally-friendly insecticidal products. The use of microencapsulation techniques leads to greater safety without any concerns towards human health, as hazardous actives are embedded within a microcapsule.

One of our formulations is Phenothrin-based microencapsulated insecticide with Prallethrin as non-microencapsulated active acting as a knockdown agent for household use. The combination of alternate classes of actives with different mode of action and the use of microencapsulation technique lead to fast killing effect of insect population. In practice, this technology - including its controlled release rate of actives with killing effect - has great potential to overcome the likelihood that an eradication programme is downgraded to long-term pest management as this factor can invoke higher economic and environmental costs. In parallel, this also allows fewer applications in order to avoid unnecessary over use of insecticides.

The release rate method represents an effective approach to describe the characteristics of the microcapsule wall in CS formulations in order to obtain controlled release properties of active ingredients.

Methodology: The release rate of actives was analysed according the OECD Test Guideline 428 (in vitro skin absorption method). Diffusion from the donor to the receptor chamber happens through a membrane with a pore size below 0.20-0.45 µm. Therefore, the microcapsules remain in the donor chamber and only the actives solubilized (or emulsified) in the water phase of the donor chamber can diffuse into the receptor chamber. The CS formulations are diluted in water and placed in the donor chamber. By stirring the donor fluid on top and the aqueous receptor fluid at the bottom, the active diffuses through the membrane filter into the receptor chamber, which is tempered at 30°C by a heating circulating system. Measurements of released actives with HPLC are done at determined intervals of time, via sampling the receptor chamber through the sampling port.

Results: As depicted in the Figure 1, the non-encapsulated Phenothrin technical and Prallethrin EW diffused very rapidly through the membrane being available after 6 h at the level of 80% in the receptor compartment.

Phenothrin microencapsulated shows an exponential but slow release with ~ 13% of release rate after 6 h, which justifies more residual efficacy with less quantity, less toxicity exposure and controlled release in order to have the necessary killing effect ,but with longer residual effect and fast killing.

Prallethrin releases much faster due to its formulation as EW following the concept to get a fast knockdown.

Conclusion: The results indicate that due to the capsule wall we have a controlled release of Phenothrin in order to reduce the quantity, which has to be available to kill the insect pests, and maintain the required amount along the time to reduce the exposure to the insecticidal product and at the same time provide an efficacy for several weeks.

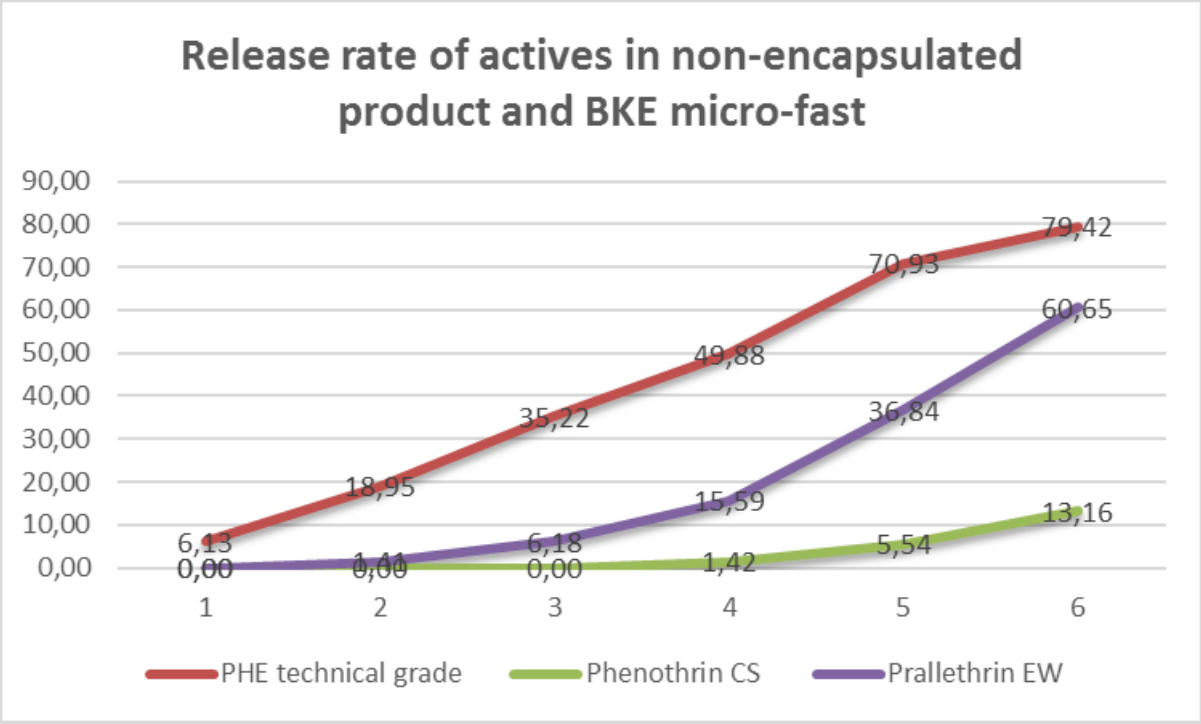


Fig. 1. *Advanced Integrated Pest Management.*

Abstract ID 200

Optimization and Use of CRISPR/Cas9 to Engineer Sperm-marked Strains of the Invasive Fruit Pest *Drosophila suzukii*

Hassan Mohammed AHMED and Ernst A. WIMMER

Department of Developmental Biology, Johann-Friedrich-Blumenbach-Institute of Zoology and Anthropology, Göttingen Center for Molecular Biosciences, Georg-August-University Göttingen, Germany, Email: *hmutasi@biologie.uni-goettingen.de*

Objectives: The objectives of the present study are to establish and optimize the genome editing tool CRISPR/Cas9 in *Drosophila suzukii* and to use the system to develop sperm-marked transgenic strains suitable for area-wide SIT programmes.

Background: The invasive fruit pest *D. suzukii* has first been detected in 2008 in Europe and the USA. Recent reports show that the pest has broadened its territories to almost all of Europe, the USA, Canada and parts of South America, which indicate, failure of current quarantine methods to contain it.

Genetic control presents itself as an effective, species specific and environmentally-friendly pest control strategy. The toolbox to generate transgenic strains suitable for area-wide biocontrol strategies has recently been enriched by the discovery of CRISPR/Cas9 as a powerful and precise genome engineering tool. Development of SIT transgenic strains necessitates the tissue- and stage-specific expression of effector molecules. The beta 2 tubulin (b2t) gene present a suitable candidate for genes specifically expressed in male gonads. Its promoter has been used for monitoring purposes in sperm-marked SIT strains or in genetic sexing in mosquitoes. Here we describe an efficient CRISPR/Cas9 genome engineering system and the isolation of the b2t gene from *D. suzukii* and its use to develop sperm-marked strains.

Methods: We used *D. suzukii* promoters of U6 genes (designated *U6A*, *U6B* and *U6C*) and Hsp 70 for transient expression of guide RNA and Cas9 respectively. We then identified an efficient gRNA that cuts upstream of the DsRed.T3 and designed marker-exchange repair template to knock-in nlsEGFPSV40 and knock-out DsRed.T3 in a transgenic strain previously generated by piggyBac germ line transformation. Based on homology to *D. melanogaster*, we identified the *D. suzukii* b2t gene. We then used a 248bp promoter region to design a marker-exchange, sperm-marking repair template. We co-injected Cas9 and gRNA plasmids with either of the two repair templates in de-chorionated pre-blastoderm embryos. Additionally, we co-injected Cas9/gRNA ribonucleoprotein along with the marker-exchange repair template.

Results: Transient expression of Cas9 and gRNA resulted in Homology Directed Repair (HDR) at a rate of 22.7% females' crosses and 9.5% males' crosses for U6C plasmid compared to 15.7% females' crosses and 7.6% males' crosses for U6A plasmid respectively.

On the other hand, injection of the ribonucleoprotein complex resulted in 33% HDR. Injection of Cas9 mix with the sperm-marking repair template resulted in expression of DsRed.T3 in the testes.

Conclusions: In the light of our results, the U6C promoter is more efficient than the U6A. The use of Cas9 protein and in vitro transcribed gRNA is the most efficient. The 5'UTR plus 51bp of b2t upstream region is sufficient to drive strong expression of DsRed.T3 in the testes. The sperm-marked strain is an enhancement for an embryonic driver strain that can be crossed to hid^Ala effector lines to produce embryonic lethality strains or crossed to sexing effector lines to produce transgenic sexing strains.

Abstract ID 201

Can Transgenic Flies Overexpressing Antioxidant Enzymes Blunt Radiation-induced Oxidative Stress, Improve Mating Success, and Inhibit Remating?

Vanessa SIMOES DIAS DE CASTRO and Daniel HAHN

University of Florida, Gainesville, Florida, USA, Email: *vanessadias@ufl.edu*

Background: The Sterile Insect Technique (SIT) is a successful autocidal biological control method that uses ionizing radiation to sterilize insects. Irradiation effectively sterilizes insects by causing dominant lethal double stranded DNA breaks, but irradiation also has side effects, including substantial oxidative stress that reduces the mating success of sterile males. Oxidative stress is a pervasive factor that can influence many aspects of organismal life histories, including reproduction.

Methodology: In this study, we hypothesize that enhancing antioxidant capacity will reduce oxidative stress and increase male mating success in the Caribbean fruit fly *Anastrepha suspensa*. By overexpressing a key antioxidant enzyme, the mitochondrial superoxide dismutase (MnSOD), in *A. suspensa*, we can test whether increasing antioxidant capacity will decrease oxidative stress and increase mating success after irradiation sterilization. We successfully produced seven transgenic lines that overexpress SOD and showed superoxide dismutase enzymatic activity up to 130% more than wild-type flies.

Results: Our results suggest that at least two SOD lines exposed to radiation had greater mating success than irradiated wild type flies. Overexpression of SOD was correlated with decreased oxidative damage to lipids but not proteins. The increment of enzymatic antioxidants in transgenic males did not affect the probability of remating of wild females, a crucial factor for the success of the SIT.

Conclusion: The results from this research can help us to better understand both the evolution of female mate choice and improve insect pest management techniques, such as the SIT.

Abstract ID 203

RapidAIM: Real-time Alerts of the Presences and Location of Fruit Fly

Nancy SCHELLHORN, Laura JONES and Darren MOORE

Commonwealth Scientific & Industrial Research Organisation (CSIRO), Brisbane, Queensland, Australia, Email: *nancy.schellhorn@csiro.au*

Background: Area-wide management of fruit fly relies on monitoring to estimate wild population size, guide targeted control for reducing populations, inform SIT release locations and rates, and determine the effectiveness of any pest control action. Furthermore, monitoring for fruit fly is mandated under trade agreements and at present it is done manually.

Millions of fruit fly traps around the globe are checked every 7-14 days. Manual checking is labour intensive, causes delays, risks fruit-fly outbreaks, and the loss of markets. We have developed rapid automated insect monitoring (rapidAIM) to provide real-time alerts of the presence and location of fruit fly.

Methodology: We've combined our knowledge of fruit fly behaviour with proprietary hardware and software to develop a grid of instrumented, low-powered smart traps, that detects the presences of a fruit fly, sends the image to the cloud for analytics and generates an alert for an end user. We have retrofitted off-the-shelf manual fruit-fly traps with our patented technology; an ultra-low-power sensor to detect the presence of an insect, and to classify whether the insect is a fruit fly.

RapidAIM is an IoT (internet of things) system – an internet-linked hardware and software system, using LPWAN (low power wide area network) with data analytic capability. We have conducted field tests demonstrating real-time detection and alerts, efficacy, and field tests comparing manual monitoring and automated real-time alerts. Technology development and testing has been conducted using laboratory reared and wild populations of *Bactrocera tryoni* (Queensland Fruit Fly), and testing is now underway with other species of fruit fly including *Ceratitis capitata* (Mediterranean fruit fly).

Results: We show that the software/hardware play detects the presence of an insect as it enters the trap, and the sensor-algorithm combination allows us to distinguish that the insect is fruit fly. This information is sent to the cloud via LPWAN where analytics are conducted and information is provided to the end user in an app including real-time, geo-referenced alerts of fruit fly, heat-maps of persistent fruit-fly activity, empty traps and early warning of future hot spots.

We show that RapidAIM is comparable to manual traps for fruit fly capture, and allows for better workflow, for example, the ability to prioritise visits to areas where fruit fly is detected, and to not waste time visiting empty traps. It is networkable and scalable; the more RapidAIM traps in a region, the better the early-warning alerts.

Conclusions: RapidAIM is a new era in bio-security and fruit-fly management. RapidAIM technology shows great promise for commercialisation, and in the future may be offered as an information service to Pest Management Crop Advisors, and Government Bio-Security agents. RapidAIM Next Gen technology, currently under development, is working towards adding functionality to the current low-power sensor to discriminate between wild-type and sterile fruit flies; a technology that can enable further efficiencies of SIT area-wide management.

Abstract ID 209

Raspberry Ketone Increases Survival and Reduces Sterile Male *Bactrocera tryoni* Froggatt Response to Cue-lure: Implications for Sterile Insect Technique Programmes

Monjur KHAN[1], Nicholas MANOUKIS[2], Geoff GURR[3], Terry OSBORNE[4], Idris BARCHIA[4] and Olivia REYNOLDS[4]

[1]Department of Entomology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]USDA-ARS, Hawaii, USA
[3]Charles Sturt University, Sydney, Australia
[4]New South Wales Government, Department of Primary Industries (NSWDPI), Australia, Email: *olivia.reynolds@dpi.nsw.gov.au*

Background: Semiochemicals are chemicals produced by one organism that incite a response in another organism. They have been used to enhance male performance of tephritids of the genera *Anastrepha*, *Bactrocera* and *Ceratitis* and include fruit or plant volatiles and oils. In Australia, the cue-lure (a derivative of the naturally occurring phenylpropanoid, raspberry ketone (4-(4-hydroxyphenyl)-2-butanone)) responding Queensland fruit fly, *Bactrocera tryoni* (Froggatt), is the most significant horticultural pest, attacking most commercial fruits and many fruiting vegetables. Among the different management options for this pest, the sterile insect technique (SIT) and cue-lure-based male annihilation technique (MAT) are regarded as two of the most effective methods, particularly when used under an Area Wide Management scenario. However, the combination of these two methods has been considered incompatible in *B. tryoni* management (as the MAT are thought to kill the sterile males).

Methodology: In the present study we tested the mortality and survival of raspberry ketone (RK)-fed adult sterile *B. tryoni* released in field cages (diet contained 0, 0.5, 1 and 2% RK) and in the field across two commercial orchards, one comprising a single stone fruit variety, the other comprising multiple stone fruit varieties (diet contained 0.2% RK), respectively. We also assessed the response of RK-fed sterile male *B. tryoni* to cue-lure in field cages and across two commercial orchards as described above.

Results: Mortality of flies in field cages was lower for RK-fed flies. Similarly, survival of flies under orchard conditions was higher for RK-fed flies than non-RK-fed flies. Data suggest a lower recapture of RK-fed sterile males than non-RK-fed sterile males in cue-lure-baited Lynfield traps in both the field cage and orchard trials. The advantage of this “male replacement” approach (mortality of wild males at lure-baited traps while simultaneously releasing sterile males) lies in the significant increase of sterile to wild male overflooding ratios, and the possibility of reducing the required number of sterile males to be released.

Conclusion: Although further research is still required, ultimately, management of *B. tryoni* could effectively combine MAT and SIT.

Abstract ID 219

Impact of Isolated Fungi *Purpureocillium lilacinum* on the Digestive Enzymes of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

Wafa DJOBBI FADHEL[1], Meriem M'SAAD GUERFALI[1], Salma KNANI[1], Kamel CHAARBI[1], Heithem HAMDEN[1], Salma FADHEL[1] and Ameur CHERIF[2]

[1]National Center for Nuclear Sciences and Technology (CNSTN), Tunisia, E-mail: *mimouniwafa87@gmail.com*

[2]LR Biotechnology and Bio-Geo Resources Valorization (LR11ES31), Higher Institute for Biotechnology - University of Manouba Biotechpole of Sidi Thabet, Ariana, Tunisia

Background: Ceratitis capitata is one of the most injurious pests of fruits with quarantine importance because of its extremely wide host range. Different methods have been used to control Mediterranean fruit fly, although essentially chemical control. However, because of the toxicity of the used pesticides, the interest for natural product-based pesticides has grown substantially.

The use of the entomopathogenic fungi constitutes a promising approach for potential application in integrated pest management. Nonetheless, developing methods of insect control can involve also the use of fungal machineries to produce metabolic disturbance that can increase its effectiveness by producing a detrimental effects on insect development.

Insect species such as *C. capitata* depend on carbohydrates for their survival. They have a role in physiological processes (maintenance, survival and reproduction). Nevertheless, lowering of carbohydrates assimilation might cause negative effects on the performance of adult flies. Accordingly, the purpose of this study is to use the entomopathogenic fungus *Purpureocillium lilacinum* to investigate its effects on the carbohydrate metabolism of *C. capitata*.

Methodology: Insects: Wild *C. capitata* were collected from infested fruits. Fungus: The entomopathogen fungus *P. lilacinum* was isolated from Tunisian green land soil. Infection assays: The pathogenicity of the fungus was tested against adult wild *C. capitata*.

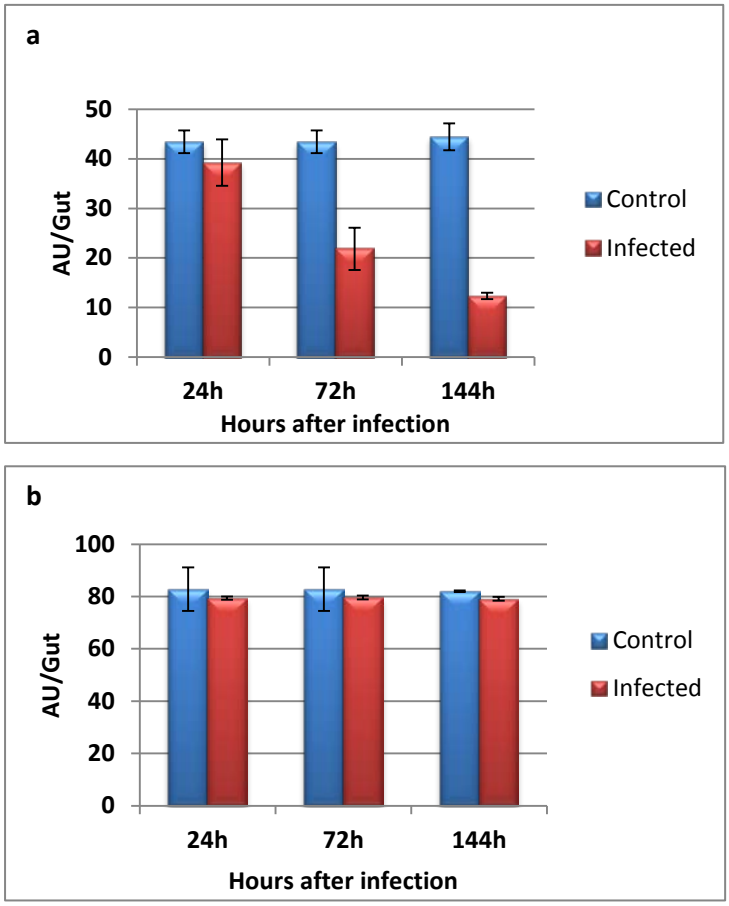
Bioassays: These were conducted using conidial concentration (1.8x106). Flies were individually treated with 5 µl of suspensions (topical application) and Tween 80 (0.02%), while the control was constituted of Tween 80 only, without fungal suspension.

Preparation of insect gut homogenates and carbohydrase assays: The activity of carbohydrase enzymes (polysaccharide and disaccharides) was determined by the Bernfeld method. One hundred and fifty adults were dissected and their guts were homogenized in Tris-HCl buffer. After the centrifugation, the supernatant was used in enzyme assays. Aliquots of homogenate were incubated with substrates (1% solutions) and sodium acetate buffer. The absorbance was measured at 540nm. All assays were performed in triplicate.

Statistical analysis: The data reported in this paper are mean values. The differences were tested using ANOVA and subsequent comparisons of the means (LSD test, at P=005).

Results: The effect of the fungus on the carbohydrates metabolism was tested against wild males and females of *C. capitata* (Figure 1). Our results show that the infection with *P. lilacinum* using the method of bioassays produces a significant change in the carbohydrase activity over time. The activity of disaccharides (pectin) and polysaccharides (starch) is significantly reduced compared to the control.

Conclusion: The work carried out in the laboratory focused on the activity of carbohydrase enzymes before and after treatment within wild medflies. In this study, we have reported that the treated flies have a significant reduction in the carbohydrase enzyme activity compared to untreated flies during the infection. This suggests the existence of an inhibition effect on the digestive system (carbohydrates metabolism) caused by the fungal strain *Purpureocillium lilacinum*. Knowing that carbohydrates play a major role in physiological processes, and especially on male sexual performance, these results can be investigated for possible use in biocontrol.



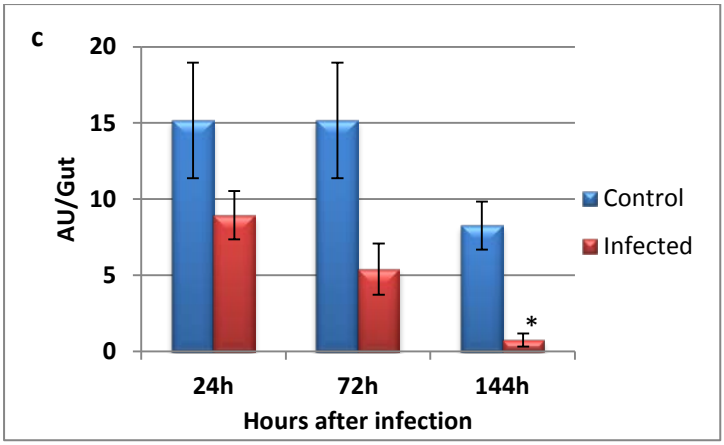


Fig. 1. *Digestive carbohydrase activity after infection of wild Ceratitis capitata by Purpureocillium lilacinum (a) Polysaccharide activity on starch; (b) Disaccharide activity on maltose; (c) Carbohydrase activity on pectin (*) indicates a significant difference between the amount of carbohydrases activities of infected and processed control flies (Fisher LSD test, p <0.05).*

Abstract ID 221

Development of a Biotechnologically Enhanced Sterile Insect Technique to Fight Coleopteran Insect Pests

Musa DAN'AZUMI ISAH, Stefan DIPPEL, Hassan Mohammed AHMED, Kolja N. ECKERMANN and Ernst A. WIMMER

Department of Developmental Biology, Johann-Friedrich-Blumenbach Institute of Zoology and Anthropology, 37077 Georg-August University, Göttingen, Germany, Email: *misah@gwdg.de*

The Sterile Insect Technique (SIT), an increasingly important component of area-wide integrated pest management is a proven and widely accepted species-specific genetic approach that acts as an insect birth control measure, which has been used successfully to prevent, suppress and eradicate populations of several insect pest species in Europe, Africa, Asia, the Americas, and Australia.

Hitherto, the application of SIT requires the mass-rearing and mass-release of sterile males using sterility usually induced by radiation or chemosterilants which are carcinogenic and also decrease the competitiveness of the sterilized males. However, transgenic approaches involving the biotechnological introduction of different types of traits into the insect strains can improve their use in SIT approaches to replace the use of radiation and chemosterilants.

Insect biotechnology provides novel strategies to improve insect pest management approaches by supplying sex-separation for male-only releases, reproductive sterility, and marking for monitoring which has being developed for many dipteran species, i.e. *Anopheles gambiae*, *Ceratits capitata*, *Drosophila* spp; and lepidopteran pests as well.

There is, however, a paucity of information of the technique being effectively transferred to coleopteran pests. Coleoptera is the largest order of the class Insecta and consist of about 220 families, which are found in almost all habitats/niches with some doing appreciable damage to both human/animal health and agricultural products both in the field and in storage.

Here we report the first proof of principle of transgenic sperm-specific marking systems based on the spermatogenesis specific β2 - tubulin (β2t), ongoing transgenic sexing system and conditional Female Embryonic Specific Lethality (FESL) using the coleopteran model insect pest *Tribolium casteneum.*

Key words: Sterile Insect Technique, transgenesis, β2-tubulin, Coleptera, Tribolium casteneum

Abstract ID 231

Distribution of Viruses Infecting Yams in Ghana and Nigeria

Chukwuemeka NKERE[1], Gabriel ATIRI[2], Joseph ONYEKA[1], Susan SEAL[3] and Lava KUMAR[4]

[1]National Root Crop Research Institute (NRCRI), Umudike, Nigeria, Email: *nkerechukwuemeka@yahoo.com*
[2]University of Ibadan, Ibadan, Nigeria
[3]NRI, Greenwich, UK
[4]IITA, Ibadan, Nigeria

Yam (*Dioscorea* spp.) is an important starchy staple crop in West Africa, providing major source of caloric energy for millions of people in this region. This region accounts for over 90% of the world's production, with Nigeria and Ghana contributing >65% of the global production.

Infection by viruses limit yam yields. Studies were conducted to assess the incidence of virus infections and virus diversity in Ghana and Nigeria in 2012 and 2013. A total of 1530 (647 from Ghana and 883 from Nigeria) infected and uninfected leaf samples from three agro-ecological zones, humid forest (HF), derived savanna (DS) and southern Guinea savanna (SGS), were purposively selected to determine percent virus disease incidence (VI) and virus disease severity using a 5-point scale, ranging from 1 (asymptomatic) to 5 (most severe mosaic symptoms on leaves).

The VI ranged from 46.7 to 100% and 26.7 to 100% in Ghana, and from 53.4 to 100% and 0.0 to 100% in Nigeria in 2012 and 2013, respectively. Using multiplex RT-PCR / PCR, the percentage samples of Ghana that tested positive were 6.7, 20.0, 61.3 and 17.1 for Yam mosaic virus (YMV), Yam mild mosaic virus (YMMV), Yam badnavirus (YBV) and mixed infections, respectively; whilst they were 6.9, 40.7, 67.5 and 38.4 in samples collected in Nigeria.

In 2013, the percentages of samples tested positive viruses were 47.9, 1.0, 93.8 and 48.2% for Ghana and 61.8, 2.6, 94.5 and 64.2% for Nigeria for YMV, YMMV, YBV and mixed infections, respectively. Cucumber mosaic virus (CMV) was not detected in any of the samples.

Our finding reveals high incidence of yam viruses in Ghana and Nigeria, possibly due to reuse of infected seed yams as planting material.

Abstract ID 245

Effect of Gamma Radiation on Electrophoretic Total Protein Patterns of Peach Fruit Fly, *Bactrocera zonata*

Nagwan ZAHRAN[1], G. M. HEGAZY[2], H. M. SALEM[1] and W. ELSAYED[2]

[1]Department of Natural Products, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Cairo, Egypt, Email: *nagwanzahran@yahoo.com*
[2]Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

The present work deals with the effect of gamma radiation on some biochemical parameters of males and females emerged from irradiated 5 days old *Bactrocera zonata* pupae.

Electrophoretic total protein patterns showed that protein of unirradiated males fractioned to 9 bands with molecular weight ranging between 66-27 k. Dalton (kDa). However, the protein bands of males emerged from irradiated pupae significantly fluctuated (disappearance and appearance of new bands). The number of bands became 9, 10, 11, 7 and 8 bands in males irradiated as 5 days old pupae with 10, 30, 50, 70 and 90 Gy, respectively.

For unirradiated females the number of protein bands detected was 9 bands with molecular weight ranging between 94-36 kDa and this number was changed after irradiation and became 8 bands at all doses. This number of bands significantly fluctuated by disappearance and appearance of new bands.

Key words: Gamma radiation, peach Fruit Fly, Bactrocera zonata*, electrophoresis protein*

Abstract ID 266

Novel Approaches to Control Invasive Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier)

Waqas WAKIL[1] and Muhammad YASIN[1,2]

[1]Department of Entomology, University of Agriculture, Faisalabad, Pakistan, Email: *waqaswakeel@hotmail.com*

[2]Department of Entomology, College of Agriculture, Sub-campus Bahaudin Zakariya University, Multan, Pakistan

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is one of the voracious species among the invasive insect pests. Here in the present studies we tested different novel approaches for the control of *R. ferrugineus*.

Initially, nineteen different isolates of *Beauveria bassiana* s.l. and *Metarhizium anisopliae* s.l. (Ascomycota: Hypocreales) were screened, to which both larvae and adults of *R. ferrugineus* exhibited varying level of susceptibility. Three isolates of *B. bassiana* and two of *M. anisopliae* exhibited highest larval and adult mortality at different exposure intervals. The capability of endophytic colonization of *B. bassiana* was assessed afterwards in live date palm petioles, and the weevil population exposed to these endophytically colonized date palm petioles was significantly reduced.

Bacillus thuringiensis var. *kurstaki* (*Bt-k*) is also an effective agent that can cause detrimental effects on *R. ferrugineus* survival alone and in combination with endophytically colonized date palms. Both agents also exerted detrimental effect on developmental parameters like larval duration, larval weight, pre-pupal duration, pre-pupal weight, pupal duration, pupal weight, adult longevity (male and female) and adult weight (male and female) etc.

Additionally, entomopathogenic fungi in integration with an entopathogenic nematode *Heterorhabditis bacteriophora* Poinar applied either simultaneously or in sequential manner exerted detrimental effects on growth and development of *R. ferrugineus* larvae.

In addition to these novel strategies, the sterile insect technique and genetically modified weevils have potential to control this insect pest when integrated with other control strategies, which will be discussed to open new horizons in this area of research in future.

Key words: Invasive species, red palm weevil, date palm, microbial control agents, SIT, Pakistan

Abstract ID 268

Chemoreception as an Adaptive Trait in the Highly Invasive Fruit Fly *Ceratitis capitata* (Diptera, Tephritidae)

Anna MALACRIDA, Giuliano GASPERI, Francesca SCOLARI and Ludvik M. GOMULSKI

Department of Biology and Biotechnology, University of Pavia, 27100 Pavia, Italy, Email: *malacrid@unipv.it*

Background: The highly invasive agricultural insect pest, *Ceratitis capitata* (Diptera: Tephritidae) is the most thoroughly studied tephritid fruit fly species, and it has become a model for the analysis of fruit fly invasions. It is an opportunistic phytophagous species whose survival and dispersion is tightly dependent on its interactions with different environments where the plasticity of its chemoreception plays a fundamental role.

Methodology and Results: The chemosensory repertoire of the Mediterranean fruit fly genome has been annotated, and a comparative analysis with the chemosensory repertoires of *Drosophila melanogaster* and *Musca domestica* revealed that the Mediterranean fruit fly is more similar to *Drosophila* than to *Musca*, perhaps because its ecology is more similar, despite being phylogenetically more basal.

Olfaction in the Mediterranean fruit fly plays a key role in regulating essential reproductive behaviours such as the detection of pheromones during the recognition and location of mates and oviposition sites. We are characterizing at the genetic, molecular, functional and structural levels the chemosensory components that regulate the complex pheromone communication system.

Conclusions: These analyses are an important step for the interpretation of the biology of the species and consequently its invasive potential. In addition, the identification of the components of the molecular machinery involved in the binding and recognition of odours and pheromones is essential for the development of powerful species- and sex-specific attractants.

Abstract ID 280

Identification and Manipulation of Symbiotic Bacteria Associated with *Bactrocera dorsalis* and *Bactrocera minax*

Awawing A. ANDONGMA, Yulei WANG, Adnan MOHAMAD, Kanjana KHAESO, Akami MAZARIN and Changying NIU

College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China, Email: *niuchangying88@163.com*

Background: Insects are associated with bacteria that affect their fitness and survival in diverse ways. These symbionts play vital roles in insect development and reproduction. In recent years, the intricate relationship between fruit flies and their symbiotic bacteria is gaining more global attention as a strategy to control pests. In this study we used *Bactrocera dorsalis* and *Bactrocera minax* as model insects to understand their bacteria community structure, effects and transmission pattern by which to pave the way for the development of efficient and sustainable pest management programmes.

Methods: Culture dependent and pyrosequencing techniques were used to identify the bacteria spectrum associated with *B. dorsalis* and *B. minax*. In addition the possible roles of isolated bacteria and their transmission patterns were studied.

Results: In *B. minax* the proteobacteria were dominant in all life stages. It was realized that egg disinfection reduced their hatching rates, but the addition of antibiotics to rearing diets had no effect on the number of copulations, longevity and fecundity. On the other hand, adding the bacteria *Enterobacter cloacae*, *Pantoea agglomerans* and *Enterococcus faecalis* significantly increased fecundity. Similarly the addition of *Klebsiella oxytoca* or *Enterococcus faecalis* to diets significantly increased the fly's life span, but this significant increase is not observed when flies were previously fed with antibiotics. Feeding flies with sugar diet had no effects on insect longevity; however, the addition of *Klebsiella oxytoca* to sugar diets significantly increased copulation and fecundity.

Regarding *B. dorsalis*, there was a shift in the dorminant bacteria group from proteobacteria in the developmental stages to Firmicutes in the adults. Continuous feeding with antibiotics significantly reduced adult fecundity and longevity. However the addition of different bacteria did not have any effects on the measured fitness parameters except *Enterococcus phoeniculicolas* that shortened the pupal developmental time and increased their survival ratio. After bacteria transformation with fluorescent proteins, *Enterobacter cloacae* and *Enterococcus faecalis* formed bacterial films in *Bactrocera dorsalis* gut 48 hours after ingestion. In addition, *Enterococcus faecalis* administered to the eggs were found in the adult gut indicating the possibility of vertical transmission.

Conclusions: Symbiotic bacteria associated with these flies play a vital role in their survival which could be exploited in their management. In addition, the relation between different fruit flies and their symbionts is complex and closely depends on a host of other factors.

Key words: Symbiotic bacteria, Bactrocera dorsalis, Bactrocera minax

This study was funded by International Atomic Energy Agency (via Research Contract CRP No. 17153) and the Joint National Science Foundation of China-Israel Science Foundation project (No. 31661143045).

Abstract ID 281

Forecasting Insect Activity Utilizing Large Network of Automated Traps and Artificial Intelligence

Matej ŠTEFANČIČ, Mateja ŠTEFANČIČ, Jernej ZUPANC, Dorian ŠUC and Boštjan BOŽIČ

Trapview, Efos Informacijske Rešitve d.o.o., Hruševje, Slovenia, Email: *matej.stefancic@efos.si*

Introduction: The authors propose a novel concept of utilizing large network of automated insect traps collecting data that are processed by advanced artificial intelligence algorithms to produce automated forecast of pest population. The aim of the concept is to provide all relevant stakeholders enough time to apply crop protection measures in the most optimal way. That ensures sustainable use of crop protection products and minimizes the risk of pest resistance.

Methods: Combining point-specific and area-wide automated pest monitoring
Point specific monitoring gives us insight into details of certain field/orchard. Historical data from the point help evaluate certain location from the pest pressure point of view and is also of key importance for forecasting future pest population development at that specific site.

In contrast to single point of monitoring, area-wide automated pest monitoring also takes into consideration some key factors influencing the decision about crop protection: crop protection measures taken, migration of pests, eliminating site-specific anomalies and statistical errors coming from a single point of monitoring.

Processing collected information with artificial intelligence algorithms
Application utilizing large sets of data (so called big data) requires accurate and reliable data. In the case of automated traps the images of caught insects need to be automatically processed by the use of computer vision algorithms.

Big Data analysis: finding patterns and rules within the data and provide forecast
Most of current research efforts of the authors are focused on experimenting with the currently most dominant trend in computer vision: **deep learning and identifying frameworks** which could be utilized to increase the accuracy of insect classification not only of an individual insect within a trap, but for maybe even different species of insects.

Machine learning is a field of Artificial Intelligence that gives computers the ability to learn without being explicitly programmed. The authors are using this approach to model the pest population dynamics and to forecast the optimal time window for the use of crop protection products. Machine learning methods are used to learn the appropriate predictive models from data collected by automated traps and manually entered data by the users.

Results: Forecasting the optimal time window for the use of crop protection product is a challenging research problem as it depends on several factors, including crop protection and

weather. As usually the crop protection is most effective in eggs hatching and larval stage of a pest population, the proper time for crop protection needs to be identified with a relatively high precision to pinpoint the exact moment within a 3 to 5 day time window. It is also challenging to asses directly what is the pest development stage as only (male) adult pest population is typically observed and counted.

These specifics make machine learning in this domain particularly difficult, requiring special care in formulating the learning problem. Another important aspect of the learning task is to be able to take into account the traps in the neighbourhood of a trap when making predictions. By using the neighbourhood and proximity of the traps allows for better predictions than by making predictions when all traps are isolated from each other and considered as single independent points. Therefore, a large network of traps provides a significant competitive advantage of automated area-wide pest monitoring.

Conclusions: A novel way of site-specific information enhanced with area wide pest monitoring using a combination of automated traps and advanced data processing has been presented. Key challenges as well as proposed solutions related to advanced image recognition and machine learning are presented. Such an approach allows more qualified crop protection decisions as it can be significantly more timely and accurate than other methods currently in use. However, in order to make such approach work a large amount of weather data combined with current and historical trap catches in networks of traps is required. Therefore, setting up a large-scale network of automated traps, which is comprised of strategically placed smaller clusters of traps, is proposed.

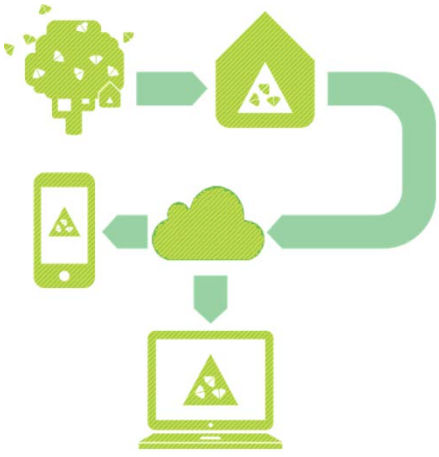


Fig. 1.*: Concept of automated pest monitoring.*

Fig. 2. *Representation of the trap network in the Mediterranean basin.*

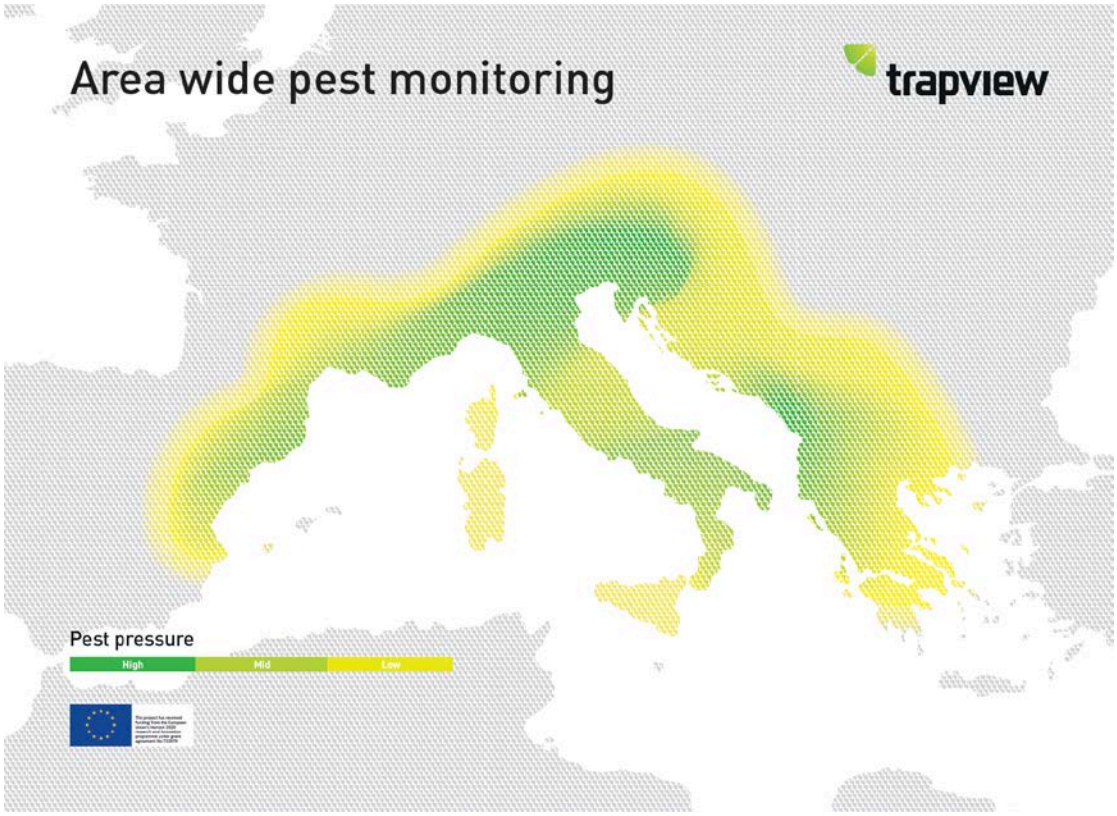


Fig. 3. *Simulation of readings from network of automated traps.*

Abstract ID 290

HOOK RPW and ISCA Smart Traps: Revolutionary New Tools for the Management of the Red Palm Weevil in Gulf countries

J.R. FALERIO[2], Abdallah Ben ABDALLAH[3], Abdulrahman S. ALDAWOOD[4], Hamadtu Abdel FARAG ELSHAFIE[5], William URRUTIA[1], Rodrigo SILVA[1], Carmem BERNARDI[1], Katherine VILLAGRAN[1], Jesse SAROLI[1] and Agenor MAFRA-NETO[1]

[1]ISCA Technologies, Inc., Riverside, CA, USA, Email: *jesse.saroli@iscatech.com*
[2]Date Palm Centre, Ministry of Agriculture, Al Hassa, Saudi Arabia, 31982
[3]Date Palm Centre, Damam Road, AL-Hassa, Saudi Arabia
[4]King Saud University, Riyadh, Saudi Arabia
[5]Hamadtu-Date Palm Research Centre, King Faisal University, Al Hassa, Saudi Arabia

The red palm weevil (RPW), *Rhynchophorus ferrugineus*, has caused significant losses to the date palm (*Phoenix dactylifera* L) industry throughout the Middle East, causing decline of fruit production and death of infested palms since its introduction into this region in the 1980s.

Monitoring of RPW is largely conducted through visual inspections of damage to date palms, and counts of RPW captured in bucket traps placed in palm fields, containing an aggregation pheromone and water with food baits. Pheromone bucket traps are difficult to maintain, requiring frequent servicing (replacement of water and food bait at least once every two weeks) in order to remain effective. As a result, compliance with the necessary trap maintenance schedule is low, and the number of traps that can be deployed within a given field is limited. Despite their shortcomings, these traps have continued to represent the standard for RPW monitoring and control for the past three decades.

We will discuss the likely effects of the introduction of two innovative technologies to the management of RPW in the Gulf region, as potential improvements upon the traditional trapping method: HOOK RPW, a semiochemical formulation that attracts and kills RPW using the standard pheromone trap without the need for food baits or water, and with a substantially longer field life. Second, the ISCA Smart Trap, a highly sensitive, accurate electronic trap capable of instantaneously transmitting capture data to the Internet for immediate analysis.

By eliminating the need for frequent servicing, HOOK RPW could strengthen current mass-trapping programmes by allowing for a higher density of trap placement in infested fields, or possibly even do away with the traps altogether, if applied as a stand-alone attract and kill formulation. ISCA Smart Traps will revolutionize the outdated data collection methods currently employed in the Gulf region by providing automated, real-time reports of the pest's activity in the field, thereby enabling faster, better informed RPW control.

International Atomic Energy Agency
IAEA-CN-248
Vienna International Centre
PO Box 100
1400 Vienna, Austria
Tel.: +43 1 2600 (0) plus extension
Fax: +43 1 26007
Email: official.mail@iaea.org

Conference website:

http://www-pub.iaea.org/iaeameetings/50813/AWConf2017

17-18531