SIP 2012

45th Annual Meeting of the Society for Invertebrate Pathology

BUENOS AIRES ARGENTINA

45th Annual Meeting of the Society for Invertebrate Pathology

2012 International Congress on Invertebrate Pathology and Microbial Control

Program and Abstracts

August 5 -9, 2012

Centro de Convenciones UCA, Puerto Madero, Buenos Aires, Argentina

2012 International Congress on Invertebrate Pathology and Microbial Control

and

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IMPORTANT NOTES:

The abstracts included in this book should not be considered to be publications and should not be cited in print without the author´s permission.

STU indicates papers being judged for graduate student presentation awards.

126 indicates abstract number for ORAL presentations

B-15 indicates abstract number for POSTER presentations

PROGRAM

SUNDAY – August 5th

SIP Council Meeting (Room 1)	**Sunday, 08:00 – 17:00**
Registration	**Sunday, 11:00 – 18:00**
Uploading powerpoint presentations	Registration Desk
Mixer (Foyer - Auditorium I)	**Sunday, 18:00 - 21:00**

MONDAY – August 6th

Registration	**Monday, 08:00 – 16:30**
Opening Ceremonies and SIP Founders´ Memorial Lecture	**Monday, 08:30 – 10:00 Auditorium 1**

Opening Ceremony

Alicia Sciocco-Cap, Chair, Local Organizing Committee
Leellen Solter, President, Society for Invertebrate Pathology
Representatives from INTA, CONICET and MINCyT
Andreas Linde, Student Awards Ceremony

Founder's Lecture

Introduction by: James J. Becnel, Chair, Founders' Lecture Committee
Honoree: Sérgio Batista Alves
Lecturer: Flavio Moscardi (Universidade Estadual de Londrina; Universidade do Oeste Paulista, Brazil) presented by Italo Delalibera (University of São Paulo School of Agriculture) on behalf of Dr. Moscardi
Progress in Microbial Pest Control in Brazil - A Tribute to Sergio Batista Alves

10:00 – 10:25 **BREAK** (Foyer Auditorium 1) Setting up Poster Session 1 (Room 3)

Plenary Symposium **Auditorium 1**
Monday, 10:30 – 12:30

Microbial Control in Public Health and Veterinary Medicine: Reality and Expectations

Organizers/Moderators: Víctor Romanowski and Alicia Sciocco-Cap

10:30 **1 Entomopathogenic fungi can change the paradigm to control blood-sucking insects: the case of Chagas disease vectors.** Nicolás Pedrini. INIBIOLP, Facultad de Ciencias Médicas, UNLP-CONICET, Argentina

11:00 **2 Use of entomopathogenic bacteria in biological control of mosquitoes and simuliids in Brazil: a critical overview.** Carlos José Pereira da Cunha Araújo-Coutinho. Laboratório de Entomologia Médica, Superintendência de Controle de Endemias, São Paulo, Brazil

11:30 **3 A bacterium against dengue: our challenge.** Luciano A. Moreira. FIOCRUZ/ Centro de Pesquisas René Rachou, Belo Horizonte, Brazil

12:00 **4 First and second generation paratransgenesis: tools for the control of global vector-borne diseases.** Ravi V. Durvasula. The Center for Global Health, Dept of Internal Medicine, University of New Mexico School of Medicine, Albuquerque, USA

12:30 – 13:50 **LUNCH** **Setting up Poster Session 1**

Afternoon Session 1 **Monday, 14:00 – 16:00**
Symposium I - Virus Division **Auditorium 2**

Viral biocontrol

Organizers: Alicia Sciocco-Cap and Marlinda L. Souza

14:00 **5 Dr. Flavio Moscardi and his relevant contribution to viral biocontrol in South America.** Marlinda L. Souza. Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Av. W5 Norte final, Brasília, DF, Brazil, CEP 70.770-900

14:30 **6 Baculovirus: research and commercialization in Colombia.** Laura Villamizar R. Biological Control Laboratory, Biotechonology and Bioindustry Center, CORPOICA, Mosquera, Colombia

15:00 **7 Application of slow-killing granuloviruses to control leaf-rollers in tea fields in Japan.** Madoka Nakai. Institute of Agriculture, Division of Bioregulation and Biointeraction. Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan 183-8509

15:30 **8 The use of *Cydia pomonella* granulovirus in organic and integrated pest management.** Johannes A. Jehle. Institute for Biological Control, Federal Research Centre for Cultivated Plants, Julius Kühn-Institut (JKI), Heinrichstraße 243, 64287 Darmstadt, Germany

Afternoon Session 2 **Monday, 14:00 – 15:00**
Workshop I - Microsporidia Division **Room 2**

Host range of Microsporidia

Organizer: Dörte Goertz

14:00 **9 Host specificity and effects of microsporidia that infect natural enemies used for biological pest control.** Susan Bjørnson. Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, Nova Scotia, Canada

Afternoon Session 3 **Monday, 15:00 – 16:15**
Contributed Papers **Room 2**

Microsporidia 1

Chair: Dörte Goertz

15:00 **10 Pathology and effects of a new microsporidium from the green lacewing, *Chrysopa carnea* used for biological pest control.** Susan Bjørnson and Thomas Steele. Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, Nova Scotia, Canada

15:15 **11 Ultrastructure and pathology of a novel microsporidian pathogen in the two-spotted ladybeetle, *Adalia bipunctata* L.** Thomas Steele and Susan Bjørnson. Biology Department, Saint Mary's University, 923 Robie Street, Halifax, NS B3H 3C3 Canada

15:30 **12 New species of spore-forming pathogens (nephridiophagids) in Malpighian tubules of insects.** Renate Radek, Daniel Wellmanns and Anja Wolf. Institute of Biology/Zoology, Free University of Berlin, Königin-Luise-Str. 1–3, 14195 Berlin, Germany

15:45 **13 Genomes of microsporidia in mosquitoes: status and preliminary findings.** James J. Becnel and Neil Sanscrainte. Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL 32608, USA

16:00 **14 Plastic parasites: extreme dimorphism in a microsporidium infecting the musculature of crabs.** G.D. Stentiford, K.B. Bateman, S.W. Feist, E. Chambers and D.M Stone. European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset DT4 8UB, United Kingdom

Afternoon Session 4 **Monday, 14:00 – 15:45**
Contributed Papers **Auditorium 4**

Bacteria 1

Chairs: Neil Crickmore and Christina Nielsen-LeRoux

14:00 **15 STU Entomopathogenic nematodes as disseminating agents for *Yersinia pseudotuberculosis*: A laboratory model.** Samuel Gengler[1,2], Anne Laudisoit[3] and Pierre Wattiau[1]. [1]Veterinary & Agrochemical Research Centre, Brussels, Belgium; [2]Institute of Life Sciences, Université Catholique de Louvain-la-Neuve (UCL), Belgium; [3]School of Biological Sciences, University of Liverpool, United Kingdom

14:15 **16 Insecticidal activity of plant root-associated Pseudomonads: Host-specific expression of the fit insect toxin.** Peter Kupferschmied[1], Maria Péchy-Tarr[1], Beat Ruffner[2], Monika Maurhofer[2] and Christoph Keel[1]. [1]Department of Fundamental Microbiology (DMF), University of Lausanne, Switzerland; [2]Plant Pathology, Institute of Integrative Biology (IBZ), ETH Zurich, Switzerland

14:30 **17 The relationships between Bt's toxic activity and population distribution.** Changlong Shu, Chunge Zhang, Lian Xu, Dafang Huang, Fuping Song, Jie Zhang. State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, P. R. China

14:45 **18 STU Screening of *cry 1* genes in *Bacillus thuringiensis* strains against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae).** [2]Arthur Augusto Gonçalves Torres,[1]Rosane Bezerra da Silva, [2]André Henrique Campelo Mourão, [1]Thais Barros Rodrigues, [2]Camila da Silva Fernandes, [3]Kátia Gisele Brasil Boregas and [3]Fernando Hercos Valicente. [1]Federal University of Lavras; [2]Federal University of São João Del Rei; [3]Embrapa Maize and Sorghum Research Station, Brazil

15:00 **19 Selection of *Bacillus thuringiensis* strains active against economically important soybean lepidopteran insects in Argentina.** Diego Sauka and Graciela Benintende. Insumos Bacterianos. Instituto de Microbiología y Zoología Agrícola (IMYZA), Instituto Nacional de Tecnología Agropecuaria (INTA). Buenos Aires, Argentina

15:15 **20 STU Characterization of naturally occurring mutations in Cry1Aa and Cry1Ac *Bacillus thuringiensis* toxins** Micheline El Khoury[1,2], Joel Chopineau[1] and Mireille Kallassy Awad[2]. [1]UMR 5253 CNRS/ENSCM/UM2/UM1, 34093 Montpellier Cedex. [2]Saint-Joseph University, Faculty of Science, Beirut, Lebanon

Afternoon Session 5 **Monday, 14:00 – 16:00**
Contributed Papers **Auditorium 3**

Fungi 1

Chairs: Claudia López Lastra and Helen Hesketh

14:00 **21 STU Assessment of environmental conditions for the successful use of *Neozygites floridana.*** Thiago Rodrigues de Castro[1], Vitalis Wafula Wekesa[2], Ingeborg Klingen[3] and Italo Delalibera Júnior[1]. [1]University of São Paulo (ESALQ), Brazil; [2]The Kenya Polytechnic University College, Kenya; [3]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway

14:15 **22 Microbial control of the sweetpotato whitefly with entomopathogenic fungi.** Hong Zhu[1, 2] and Jeong Jun Kim[1]. [1]Agricultural Microbiology Team, National Academy of Agricultural Science, Suwon, 441-707, Rep. of Korea, [2]Key Laboratory of Microbial Control, Anhui Agricultural University, Hefei 230036, People's Republic of China

14.30 **23 STU *Beauveria brongniartii* epizootics on white grubs attacking sugarcane in South Africa.** Tarryn Anne Goble [1, 3], Laurent Costet L [4], Isabelle Robene [4], Samuel Nibouche [4], Stuart Rutherford [1], Desmond Conlong [1, 2] and Martin Hill[3]. [1]South African Sugarcane Research Institute, 170 Flanders Drive, Mount Edgecombe, 4300, South Africa; [2]School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg Campus, John Bews Building, Scottsville, 3209, South Africa; [3]Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa; [4]CIRAD – UMR PVBMT, F-97410 Saint Pierre, Réunion, France

14:45 **24 STU Potential of entomopathogenic fungi as bed bug control agents.** Alexis M. Barbarin[1], Nina E. Jenkins[1], Edwin G.' Rajotte[1] and Matthew B. Thomas[1,2]. [1]Department of Entomology, Penn State University, 501 Agricultural Sciences & Industries Building, University Park, PA 16802, USA; [2]Center for Infectious Disease Dynamics, Penn State University, 112 Merkle Lab, University Park, PA 16802, USA

15:00 **25 Development of strategies for the incorporation of mycopesticides into the integrated management of *Diaphorina citri* (Hemiptera: Psyllidae).** Italo Delalibera Jr., Celeste P. D'Alessandro, Marcos R. Conceschi and John J. Saldarriaga Ausique. Department of Entomology and Acarology, ESALQ, University of São Paulo, Av. Pádua Dias 11, C.P. 9, Piracicaba, São Paulo, Brazil

15:15 **26 *Isaria fumosorosea* for control of fruit moths: Comparison of submerged spores and aerial conidia.** Dietrich Stephan. Julius Kühn-Institut, Institute for Biological Control, Heinrichstrasse 243, 64287 Darmstadt, Germany

15:30 **27 Selection of promising fungal biological control agent of the western flower thrips *Frankliniella occidentalis* and development of application strategy.** S. Niassy[1], S. Subramanian[1], S. Ekesi[1], L.M. Gitonga[2], D.M. Mburu[1], D. Masiga[1] and N.K. Maniania[1]. [1]International Centre of Insect Physiology and Ecology (*icipe*), P.O. Box 30772-00100, Nairobi, Kenya; [2]Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62000, Nairobi, Kenya

15:45 **28 Comparison of microsclerotia production by various *Metarhizium* species.** Mark A. Jackson[1] and Stefan T. Jaronski[2]. [1]USDA-ARS, National Center for Agricultural Utilization Research, 1815 N University St, Peoria, Illinois, USA. [2]USDA-ARS, Pest Management Research Unit, Northern Plains Agricultural Research Laboratory, 1500 N. Central Avenue, Sydney, Montana, 59270, USA

Afternoon Session 5 **Monday, 14:00 – 15:30**
Contributed Papers **Room 1**

Nematodes 1

Chairs: M. Fernanda Achinelly and Ed Lewis

14:00 **29 Mass culturing *Steinernema yirgalemense* using *in vitro* liquid technology.** Tiarin Ferreira and A.P. Malan. Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

14:15 **30 Slug parasitic nematodes for biocontrol of the invasive slug *Arion vulgaris.*** Solveig Haukeland[1], Karin Westrum[1], Marcin Grabowski[2] and May-Bente Brurberg[1]. [1]Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Høgskoleveien 7, 1432 Ås, Norway; [2]Department of Applied Entomology, Warsaw University of Life Sciences, Nowoursynowska St. 159, 02-776 Warsaw, Poland

14:30 **31 Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae): Efficacy against *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) in Georgia.** Nona V. Mikaia. Department Natural Faculty and Health Care, Sokhumi State University, 9 Anna Politkovskaya Str.,0186 Tbilisi, Georgia

14:45 **32 Virulence of entomopathogenic nematodes to larvae of the guava weevil, *Conotrachelus psidii* (Coleoptera: Curculionidae).** Clara Delgado and Adriana Sáenz Aponte. Unit of Ecology and Systematics –UNESIS, Biological Control Laboratory, Pontificia Universidad Javeriana, Cra. 7 N° 43-82, place 54, Of 200. Bogotá, Colombia

15:00 **33 Control of *Conotrachelus psidii* (Coleoptera: Curculionidae) with insect cadavers of *Heterorhabditis* sp1. SL0708 (Nematoda: Rhabditida).** Clara Delgado[1] and Adriana Sáenz Aponte[2]. [1]Javeriana. Bogotá, Colombia [2] Unit of Ecology and Systematics –UNESIS, Biological Control Laboratory, Pontificia Universidad Javeriana, Cra 7 N° 43-82, place 54, Of 200. Bogotá, Colombia

15:15 **34 Compatibility between entomopathogenic nematodes and a neem-based product.** Elder S. P. Batista, Ana Carolina P. Veiga, Crislany L. Barbosa, Nara E. L. Rodrigues, Ricardo A. Calore and Ricardo A. Polanczyk. Unesp/FCAV, Jaboticabal Campus, Brazil

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16:00 – 16:25 **BREAK** **Setting up Posters Session 1 (Room 3)**

Poster Session 1
Bacteria

Monday, 16:30 – 18:30
Room 3

B-01 **STU** **Interaction between Cry1Ia and Vip3Aa proteins from *Bacillus thuringiensis* towards larvae of *Spodoptera* spp. (Lepidoptera).** Vivian Boter Bergamasco[1], Deise Reis de Paula Mendes[1], Odair Aparecido Fernandes[2], Janete Apparecida Desidério[1] and Manoel Victor Franco Lemos[1]. Faculty of Agronomic and Veterinary Sciences - FCAV, São Paulo State University - UNESP, [1]Department of Applied Biology, [2]Department of Plant Protection. Via de Acesso Prof. Paulo Donato Castellane, s/n, CEP14884-900, Jaboticabal, São Paulo, Brazil

B-02 **Overexpression of the toxin Cry10Aa in a wild-type *Bacillus thuringiensis* svar. *israelensis* strain.** M. Cristina Del Rincón-Castro[1], Eréndira Hernandéz-Guillén[1] and Jorge E. Ibarra[2]. [1]Departamento de Alimentos, División de Ciencias de la Vida, Universidad de Guanajuato. Km. 9.0 Libram. Norte. Carr. Irapuato-León, 36500 Irapuato, Guanajuato;. [2]CINVESTAV Unidad Irapuato. Km. 9.6 Libram. Norte. Carr. Irapuato-León, 36500 Irapuato, Guanajuato; Mexico

B-03 **Effect of Hexanoic acid plant treatment on Cry3Aa toxicity against CPB.** Inmaculada García-Robles, Carolina Rausell and Maria Dolores Real. Departamento de Genética, Universidad de Valencia, Burjassot, Spain

B-04 **STU** **Two cadherin repeat containing proteins are Cry3Ba toxin functional receptors in *T. castaneum.*** Estefanía Contreras[1], Michael Schoppmeier[2], Maria Dolores Real[1] and Carolina Rausell[1]. [1]Department of Genetics, University of Valencia, Dr. Moliner 50, 46100-Burjassot (Valencia), Spain; [2]Department of Biology, Developmental Biology Unit, University of Erlangen-Nürnberg, Staudtstr. 5, 91058-Erlangen, Germany

B-05 **Cry3Aa toxin interacts with Colorado potato beetle prohibitin-1, an essential protein for larval viability.** Camila Ochoa-Campuzano, María Dolores Real and Carolina Rausell. Departamento de Genética, Facultad de Ciencias Biológicas, Universidad de Valencia, Dr. Moliner 50, Burjassot 46100, Valencia, Spain

B-06 **STU** ***Spodoptera exigua* lectins: a protein family involved in the immune response to different pathogens.** Laila Gasmi, Agata K. Jakubowska, Juan Ferré and Salvador Herrero. Laboratory of genetics, biotechnology and biochemistry, Department of genetics, Universitat de València 46100 –Burjassot (Valencia), Spain

B-07 **Degenerate PCR based search for *cry* genes and characterization of novel *cry* genes from *Bacillus thuringiensis.*** Yu Karatani, Hiromi Hadano, Jun Makimoto, Yurika Kubo, Yuta Sugimori, Yoshinao Azuma, and So Takebe. Faculty of Biology-Oriented Science and Technology, Kinki University, Wakayama 649-6493, Japan

B-08 **STU** **Endophytic colonization by Brazilian strains of *Bacillus thuringiensis* on cabbage seedlings to control *Plutella xylostella.*** Lílian B. Praça, Glaúcia B. Cabral, Carla F. Caixeta, Ana Cristina M. M. Gomes and Rose G. Monnerat. Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Brasilia, Brazil

B-09 **STU** **Putative loop 1 in domain II of *Bacillus thuringiensis* Cry39Aa toxin are important for larvicidal activity against *Anopheles stephensi.*** Shun-ichiro Ishigaki, Hisanori Bando and Shin-ichiro Asano. Graduate School of Agriculture, Hokkaido University, N9 W9, Sapporo, 060-8589, Japan

B-10 **The adaption evolution and distribution analysis of *cry1I* gene in Bt strain.** Chan Zhao, Changlong Shu, Chunge Zhang, Dafang Huang, Fuping Song and Jie Zhang. State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, P. R. China

B-11 **Effects of Vip3Aa and Cry1Ac on enzyme activities in larvae of cotton bollworm *Helicoverpa armigera.*** Yan Zhang, Yanhui Lu, Zhen Gao and Gemei Liang. State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

B-12 **Identification of *cry* genes from *Bacillus thuringiensis* isolates with potential for control of *Ecdytolopha aurantiana* (Lima, 1927) (Lepidoptera: Tortricidae).** Ana P. S. Ricieto[1], Ana M. Menequim[2], Fernanda A. P. Fazion[1], Pamella C. Souza[2], Laurival A. Vilas-Bôas[1] and Gislayne T. Vilas-Bôas[1]. [1]Universidade Estadual de Londrina–Departamento de Ciências Biológicas. [2]Instituto Agronômico do Paraná, Brazil

B-13 **Characterization of *Bacillus thuringiensis* isolates with toxic activity against economically important insect pests in Brazil.** Josiane A. Scarpassa[1], Kelly C. Constanski[1], Pedro M. O. J Neves[1], Flávio Moscardi[1;2], Fabiane Cunha[2], Laurival A. Vilas-Boas[1] and Gislayne T. Vilas-Bôas[1]. [1]State University of Londrina, 86051-970 - Londrina, PR, Brazil; [2]UNOESTE, Presidente Prudente, SP, Brazil

B-14 **STU** **Relationship between crystal shape and fingerprinting (rep-PCR) of the *Bacillus thuringiensis.*** Thais Barros Rodrigues[1], Rosane Bezerra da Silva[1], André Henrique Campelo Mourão[2], Arthur Augusto Gonçalves Torres[2], Camila da Silva Fernandes[2], Kátia Gisele Brasil Boregas[3] and Fernando Hercos Valicente[3]. [1]Federal University of Lavras; [2]Federal University of São João Del Rei; [3]Embrapa Maize and Sorghum Research Station, Brazil

B-15 **STU** **Detection of genes *cry 2* and *cry 9* in strains of *Bacillus thuringiensis* for the control of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae).** [1]Rosane Bezerra da Silva, [2]Arthur Augusto Gonçalves Torres, [1]Thais Barros Rodrigues, [2]André Henrique Campelo Mourão, [2]Camila da Silva Fernandes, [3]Kátia Gisele Brasil Boregas and [3]Fernando Hercos Valicente. [1]Federal University of Lavras; [2]Federal University of São João Del Rei; [3]Embrapa Maize and Sorghum Research Station, Brazil

B-16 **Molecular characterization and production of *Bacillus thuringiensis* based biopesticide.** André Henrique Campelo Mourão[1], Rosane Bezerra da Silva[2], Camila da Silva Fernandes[1], Thais Barros Rodrigues[2], Arthur Augusto Gonçalves Torres[1], Kátia Gisele Brasil Boregas[3] and Fernando Hercos Valicente[3]. [1]Federal University of São João Del Rei; [2]Federal University of Lavras; [3]Embrapa Maize and Sorghum Research, Brazil

B-17 **STU** **Isolation, diversity, cloning and molecular characterization of cry gene contents from *Bacillus thuringiensis* isolates.** H.M. Mahadeva Swamy[1], R. Asokan[1], A.S. Sidhu[1], Riaz Mahmood[2] and Dilip K. Arora[3]. [1]Indian Institute of Horticultural Research (IIHR), Hessaraghatta Lake Post, Bangalore 560089 Karnataka; [2] Post-Graduate Department of Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Jnanasahayadri, Shankaraghatta, Shimoga 577451 Karnataka; [3] National Bureau of Agriculturally Important Micro Organisms (NBAIM), Mau Nath Bhanjan, 275101 Uttar Pradesh, India

Poster Session 1
Diseases of Beneficial Invertebrates

Monday, 16:30 – 18:30
Room 3

DBI-01 **Parasitic castration of a marine snail by larval trematodes**. Pilar Alda and Sergio R. Martorelli. Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CONICET-CCT La Plata-UNLP, Calle 2 No. 584, 1900, La Plata, Buenos Aires, Argentina

DBI-02 **STU** **Immunity related genes in honey bees in response to synthetic acaricidal treatments.** Paula Melisa Garrido[1], Karina Antúnez[2], Mariana Martín[3], Martín Pablo Porrini[1]and Martín Javier Eguaras[1]. [1]Laboratorio de Artrópodos Facultad de Ciencias Exactas y Naturales. Universidad Nacional de Mar del Plata - CONICET, Mar del Plata, Buenos Aires, Argentina; [2]Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; [3]Centro de Investigaciones Biológicas, CEBB-MdP-INBA, Fundación para Investigaciones Biológicas Aplicadas (FIBA), Mar del Plata, Argentina

DBI-03 **The fatal relationship between *Varroa destructor*, DWV, and honey bees.** Sebastian Gisder, Caspar Schöning and Elke Genersch. Institute for Bee Research, Dept. for Molecular Microbiology and Bee Diseases, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany

DBI-04 **Changes and similarities in the expression of honey bee immune response genes during the infection with two different genotypes of the bee pathogen *Paenibacillus larvae.*** Gillian Hertlein, Eva Garcia-Gonzalez, Sebastian Gisder, Lena Poppinga, Anne Fünfhaus and Elke Genersch. Institute for Bee Research Hohen Neuendorf, Division of Diagnostic and Molecular Biology, Friedrich-Engels-Str. 32, D-16540 Hohen Neuendorf, Germany

DBI-05 **Infectious agents of *Litopenaeus vannamei* (Boone, 1931) and their relationship with physicochemical parameters in three different culture systems in Gulf of Mexico, Mexico.** Zinnia Judith Molina-Garza[1], Gilberto Gutiérrez-Salazar[2], Mario Hernández-Acosta[3], Roberto Mercado-Hernández[1] and Lucio Galaviz-Silva[1]. [1]Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Ave. Universidad, SN, Cd. Universitaria, San Nicolás de los Garza, Nuevo León, CP.66451, México; [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Carretera Victoria-Mante Km 5, Cd. Victoria, Tamaulipas, CP.87000, México; [3]Universidad Tecnológica del Mar de Tamaulipas Bicentenario, La Pesca, Tamaulipas, CP. 87678, México

DBI-06 ***Apis mellifera* cellular immune responses *in-vitro*: Qualitative differences before and after the pupae metamorphosis black box.** Pedro Negri[1,3], Matias Daniel Maggi[1,3], Natalia Fernandez[1,3], Lorenzo Lamattina[2,3] and Martin Javier Eguaras[1,3]. [1]Laboratorio de Artrópodos, Universidad Nacional de Mar del Plata; [2]Instituto de Investigaciones Biológicas-CONICET, Universidad Nacional de Mar del Plata; [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

DBI-07 **Survey for *Nosema* spp. in Belize Apiaries.** Brenna Traver[1], Glen N.Stevens[2], Juliana Rangel[3], Mario Howe[4] and Richard Fell[1]. [1]Virginia Tech, Department of Entomology, Blacksburg, VA 24061 USA; [2]Ferrum College, Department of Biology and Environmental Science, Ferrum, VA 24088 USA; [3]North Carolina State University, Department of Entomology, Raleigh, NC 27695 USA; [4]Agriculture Department Extension Service, Central Farm, Cayo District, Belize C.A.

DBI-08 **STU Evaluation of the toxicity of essential oil components on *Varroa destructor* (Acari: Varroidae) and *Apis mellifera* (Hymenoptera: Apidae).** Constanza Brasesco[1], Matías Daniel Maggi[1,2], Pedro Negri[1.2], Liesel Gende[1.2], Sergio Ruffinengo[3], Nicolás Szawarski[1] and Martín Eguaras[1.2]. [1]Laboratorio de Artrópodos, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata; [2]Consejo Nacional de Investigaciones Científicas y Técnicas; [3]Apicultura. Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina

Poster Session 1
Fungi

Monday, 16:30 – 18:30
Room 3

F-01 ***In vitro* activity of *Laurus nobilis*, *Calamintha officinalis* and *Lippia alba against Ascosphaera apis.* Evaluation of the potential toxic effects on adults and larvae of *Apis mellifera.*** Sebastián Rodríguez[1], Francisco Reynaldi[2,4], Jorge Ringuelet[3], Susana Córdoba[4] and Graciela Albo[1]. [1]Producción Animal. Facultad de Ciencias Agrarias y Forestales. Universidad Nacional de La Plata. [2]CONICET CCT. La Plata; [3]Bioquímica y Fitoquímica. Facultad de Ciencias Agrarias y Forestales. UNLP; [4]Micología Médica e Industrial. Facultad de Ciencias Veterinarias. UNLP, La Plata, Argentina

F-02 **Effect of *in vitro* successive subcultures of *Beauveria bassiana* to U.V tolerance.** Janaina Zorzetti[1], Patricia H. Santoro[1], Kelly C. K. Silva[1] and Pedro M. O. J. Neves[1] .[1]Agronomy Department, Microbial Insects Control Laboratory, State University of Londrina, 86051-970 - Londrina, Paraná, Brazil

F-03 **Influence of the nutritional conditions on *Beauveria bassiana* (Bals.) Vuill. tolerance to temperature.** Janaina Zorzetti[1], Patricia H. Santoro[1], Kelly C. K. Silva[1] and Pedro M. O. J. Neves[1]. [1]Agronomy Department, Microbial Insects Control Laboratory, State University of Londrina, 86051-970 - Londrina, Paraná, Brazil

F-04 **Isolation of *Metarhizium* spp. from roots of different crops: Are specific genotypes associated with certain plants?** Bernhardt M. Steinwender[1], Jürg Enkerli[2], Michael J. Bidochka[3], Franco Widmer[2], Jørgen Eilenberg[1] and Nicolai V. Meyling[1]. [1]Department of Agriculture and Ecology, Faculty of Sciences, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frederiksberg C., Denmark; [2]Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, 8046 Zürich, Switzerland; [3]Department of Biology, Brock University, St. Catharines, ON Canada L2S 3A1

F-05 **STU** **Conidial water affinity is an important characteristic for thermotolerance in entomopathogenic fungi.** Roberta Kelly de Faria Souza, Rosana de Fátima Faria Azevedo and Drauzio Eduardo Naretto Rangel. Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP 12244-000, Brazil

F-06 **STU** **Tolerance of entomopathogenic fungi to oxidative stress.** Rosana de Fátima Faria Azevedo, Roberta Kelly de Faria Souza and Drauzio Eduardo Naretto Rangel Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP 12244-000, Brazil

F-07 **Antimicrobial and antioxidant activity of culture supernatant of entomopathogenic fungi.** Tae Young Shin, Won Woo Lee, Jae Bang Choi, Sung Min Bae, Yeon Ho Je[1], Byung Rae Jin[2] and Soo Dong Woo. Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; [1]School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, Korea; [2]College of Natural Resources and Life Science, Dong-A University, Busan, Korea

F-08 **The autophagy related gene, *ATG5*, affects conidia yield, morphology, germination and pathogenisis in entomopathogen *Beauveria bassiana*.** Sheng-Hua Ying and Ming-Guang Feng. Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou 310058, P.R. China

F-09 **STU** **Host-dependent lineage diversification of Scarabaeidae-specific pathogen *Metarhizium majus*.** Oumi Nishi[1,2], Kazuhiro Iiyama[1], Chisa Yasunaga-Aoki[1] and Susumu Shimizu[1]. [1]Laboratory of Insect Pathology and Microbial Control, Institute of Biological Control, Kyushu University; [2]Japan Society for the Promotion of Science. Japan

F-10 **Evaluation of two *Metarhizium anisopliae* for control of drill Paraguay tea *Hedypathes betulinus* (Klug) adults (Coleoptera: Cerambycidae).** Maria Elena Schapovaloff[1], André Luis Fanti[2], Luis Francisco Alves[2], Maria Inés Urrutia and Claudia Cristina López Lastra[1]. [1]Laboratorio de Hongos Entomopatógenos. Centro de Estudios Parasitológicos y de Vectores. CEPAVE. Universidad Nacional de La Plata. UNLP. Calle 2 N° 584 (1900). La Plata, Buenos Aires. Argentina; [2]Laboratorio de Biotecnología Agrícola. Universidade Estadual do Oeste do Paraná. *Campus* UNIOESTE. Cascavel, Paraná. Brasil; [3]Centro Superior para el Procesamiento de la Información (CeSPI-UNLP), La Plata, Buenos Aires, Argentina

F-11 **Identification and phylogenetic analysis of Brazilian strains of *Metarhizium anisopliae s.l.*** Janayne M. Rezende[1,2], Mariana da S. Lopes[1] and Italo Delalibera Jr.[1]. [1]Department of Entomology and Acarology, ESALQ, University of São Paulo, Piracicaba, São Paulo, Brazil

F-12 ***Beauveria bassiana* infection alters reproductive parameters of the Chagas disease vector *Triatoma infestans*.** Lucas Forlani, Nicolás Pedrini and M. Patricia Juárez. Instituto de Investigaciones Bioquímicas de La Plata, Facultad de Ciencias Médicas (UNLP), Calles 60 y 120, La Plata, Argentina

F-13 **Insect cuticular lipid degradation: characterization of cytochrome P450 monooxygenases from the entomopathogenic fungus *Beauveria bassiana*.** Carla Huarte Bonnet[1], Shizhu Zhang[2], Nemat O. Keyhani[2], M. Patricia Juárez[1] and Nicolás Pedrini[1]. [1]Instituto de Investigaciones Bioquímicas de La Plata, Facultad de Ciencias Médicas (UNLP), Calles 60 y 120, La Plata, Argentina; [2]Dept. of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611, USA

F-14 **Root colonizer and endophytic abilities of entomopathogenic *Lecanicillium* spp.** Masanori Koike and Daigo Aiuchi. Department Agro-Environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

F-15 **STU** **Comparing pathogenicity and infectivity of anamorphic entomopathogenic fungi isolated from the whole or inside of wild mosquito body against adult female *Anopheles stephensi*.** Minehiro Ishii[1], Junya Takeshita[1], Mitsugu Ishiyama[1], Shinya Fukumoto[2], Hirotaka Kanuka[3], Masanori Koike[1] and Daigo Aiuchi[2]. [1]Department of Agro-environmental Science, Obihiro University of Agriculture &Veterinary Medicine, Obihiro, Hokkaido 080-8555; [2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine; Obihiro, Hokkaido 080-8555; [3]Department of Tropical Medicine, Jikei University School of Medicine, Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan

F-16 **Effect of temperature on radial growth of *Beauveria bassiana* and *Metarhizium anisopliae* isolates pathogenic to boll weevil, *Anthonomus grandis*.** Ana Laura Nussenbaum and Roberto Lecuona. Laboratorio de Hongos Entomopatógenos IMyZA, CICvyA, INTA Castelar, Buenos Aires, Argentina

F-17 **Preliminary study in the selection of *Metarhizium anisopliae* isolates for microbial control of "stable fly" (*Stomoxys calcitrans*) and "house fly" (*Musca domestica*) in dairy.** Maricel Angulo Lewylle, Ana Laura Nussenbaum and Roberto Eduardo Lecuona. Laboratorio de Hongos Entomopatógenos IMyZA, CICVyA. INTA Castelar, Buenos Aires, Argentina

F-18 **Field applications of entomopathogenic fungi to control of *Diaphorina citri* (Hemiptera: Psyllidae) in Brazil.** Marcos R. Conceschi, Celeste P. D'Alessandro, John J. Saldarriaga Ausique and Italo Delalibera Jr. Department of Entomology and Acarology, ESALQ, University of São Paulo, Av. Pádua Dias 11, C.P. 9, Piracicaba, São Paulo, Brazil

F-19 **Occurrence and distribution of insect pathogenic soil fungi in agro- and forest ecosystem in Eastern Georgia.** Medea Burjanadze[1], Mariam Arjevanidze[1], Giuli Tsereteli[1], Manana Lortkipanidze[2], Cezary.Tkaczuk[3] and Jørgen Eilenberg[4]. [1] Agricultural University of Georgia, Vasil Gulisashvili Forest institute, [2]Ilis State University, Institute of Zoology, [3] University of Podlasie, Poland, [4] University of Copenhagen, Denmark

Poster Session 1
Microbial Control

Monday, 16:30 – 18:30
Room 3

MC-01 **Study on the characteristics and pathogenicity of the *Beauveria bassiana* as a control agent of *Hyphantria cunea* (Lepidoptera: Arctiidae).** Medea Burjanadze[1], Mariam Arjevanidze[1], Iamze Kaladze[1], Elena Nakaidze, Tea Abramishvili[1] and Stefan Jaronski[2]. [1]Agricultural University of Georgia, Vasil Gulisashvili Forest institute, Tbilisi, Georgia; [2]USDA ARS Northern Plains Agricultural Research Laboratory, Sidney MT USA

MC-02 **STU Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* to the legume pod borer, *Maruca vitrata* and the performance of two candidate isolates in four liquid culture media.** Venansio Tumuhaise[1,2], Sunday Ekesi[1], Samira F. Mohamed[1], Paul N. Ndegwa[2], Lucy W. Irungu[2]and Nguya K. Maniania[1]. [1]International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772 - 00100, Nairobi, Kenya, [2] University of Nairobi, P. O. Box 30197 - 00100, Nairobi, Kenya

MC-03 **Preliminary study of *Metarhizium anisopliae* production on bioreactor: concentration of inoculums.** Vivian Amanda F. Costa[1], Fabrício M. Buriola[1], Adriana Regina Generoso[3], Mariana Taglietto de Oliveira[2] and Cesar de O. Guimarães[4]. [1] Technologist in Agribusiness; [2] Undergraduated student of Technology in Agribusiness of the Faculty of Technology of São José do Rio Preto - FATEC, Brazil; [3] Professor in FATEC, Brazil; [4] Oligos Biotecnologia

MC-04 **Influence of treatment interval between eco-friendly agricultural products and *Beauveria bassiana* GHA for sweetpotato whitefly control.** Ji Hee Han, Jeong Jun Kim, Do Yeun Kim and Sangyeob Lee. Agricultural Microbiology Team, National Academy of Agricultural Science, Suwon, 441-707, Rep. of Korea

MC-05 **First record of the genus *Protomagalhaensia* (Eugregarinida: Hirmocystidae) Pinto, 1918 in cockroaches from Argentina.** Alejandra C. Gutierrez [1,2], Mariana Dellapé[1], Claudia C. López Lastra[1,3] and Juan J. García[1,2]. [1]Centro de Estudios Parasitológicos y de Vectores (CEPAVE); [2](CIC-UNLP); [3](CONICET-UNLP). Calle 2 Nº 584, CP 1900, La Plata, Buenos Aires, Argentina

MC-06 **Detection and imaging of *Metarhizium* infection of wireworms using antibodies and electron microscopy.** Todd Kabaluk[1], Claudia Sheedy[2], Grant Duke[2] and Frances Leggett[2]. Agriculture and Agri-Food Canada; [1]Agassiz, British Columbia; [2]Lethbridge, Alberta, Canada

MC-07 **Screening and evaluation of entomopathogenic fungi to the green peach aphid, *Myzus persicae.*** Won Woo Lee, Tae Young Shin, Jae Bang Choi, Sung Min Bae, Yeon Ho Je[1], Byung Rae Jin[2] and Soo Dong Woo. Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; [1]School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, Korea; [2]College of Natural Resources and Life Science, Dong-A University, Busan, Korea

MC-08 **Efficacy of entomopathogenic hypocrealean fungi to *Periplaneta americana.*** Rayssa F. Hubner-Campos, Renan N Leles, Juscelino Rodrigues and Christian Luz. DMIPP, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia, GO, Brazil

MC-09 **A new formulation of *Metarhizium anisopliae* against *Triatoma infestans.*** Juscelino Rodrigues, Luiz FN Rocha, Flávia R da Paixão and Christian Luz. DMIPP, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia, GO, Brazil

MC-10 **Decreased viability of the hemocytes of *Galleria mellonella* larvae under the *Habrobracon hebetor* venom.** Natalia A. Kryukova[1], Ekaterina A.Chertkova[1], Viktor V. Glupov[1] and Irina A. Slepneva[2]. [1] Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia; [2] Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

MC-11 ***Metarhizium anisopliae*** **for the control of** ***Aedes aegypti.*** Luciana S. Lobo, Nathália A. Sousa, Priscilla R. Borges, Juscelino Rodrigues, Éverton K.K. Fernandes and Christian Luz. DMIPP, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia, GO, Brazil

MC-12 **Virulence of Brazilian isolates of entomopathogenic fungi against different life stages of** ***Bemisia tabaci*** **biotype B (Hemiptera: Aleyrodidae).** Gabriel Moura Mascarin[1,2], Nilce Naomi Kobori[1], Eliane Dias Quintela[1] and Italo Delalibera Jr[2]. [1]EMBRAPA Rice and Beans, Rodovia GO-462, Km 12, Zona Rural, C.P. 179, 75375-000, Santo Antônio de Goiás – GO, Brazil;. [2]Department of Entomology and Acarology, ESALQ, University of São Paulo. Av. Pádua Dias, 11, C.P. 9, CEP 13418-900, Piracicaba – SP, Brazil

MC-13 **Enhanced susceptibility of** ***Tibraca limbativentris*** **(Heteroptera: Pentatomidae) to** ***Metarhizium anisopliae*** **with sublethal doses of chemical insecticides.** Eliane Dias Quintela[1], Rodrigo Alves da Silva[1], Gabriel Moura Mascarin[1], José Alexandre Freitas Barrigossi[1] and Luciano Moraes Lião[2]. [1]EMBRAPA Arroz e Feijão, Rodovia GO-462, Km 12, Zona Rural, C.P. 179, 75375-000, Santo Antônio de Goiás – GO, Brasil. [2]Universidade Federal de Goiás, Campus Samambaia, C.P. 131, 74001-970, Goiânia – GO, Brasil

MC-14 **Development and testing of formulations of** ***Lecanicillium*** **spp. for the biological control of white flies.** Federico Rivas[1], Trevor Jackson[2], Nora Altier[1], Noelia Casco[1], Jayanthi Swaminathan[2] and Tracey Nelson[2]. [1]National Institute for Agricultural Research (INIA), Las Brujas – Canelones, Uruguay; [2]AgResearch (AgR), Lincoln - Christchurch, New Zealand

MC-15 **Combined use of** ***Steinernema brazilense*** **with** ***Beauveria bassiana*** **against the sugarcane billbug,** ***Sphenophorus levis*** **(Coleoptera: Curculionidae).** Lucas Detogni Simi[1,3], Luis Garrigós Leite[2], Renata Marraschil[2], Fernanda Polastre Pereira[2], Mariana Garcia Martinez-Silva[2], Ana Paula Santos-Bartels[2], Roselaine Nunes da Silva Bueno[2] and Antonio Batista Filho[2]. [1]Faculdade de Ciências Agronômicas/Universidade Estadual Paulista - Depto. de Produção Vegetal / Defesa Fitossanitária, Botucatu, São Paulo, Brazil; [2]Instituto Biológico - Laboratório de Controle Biológico, Campinas, São Paulo, Brazi; [3]CNPq, Brazil

MC-16 **Preliminary study of** ***Metarhizium anisopliae*** **production on bioreactor: use of inert with rice as substract.** Fabrício M. Buriola[1], Mariana Taglietto de Oliveira[2], Adriana Regina Generoso[3] and Cesar de O. Guimarães[4]. [1]Technologist in Agribusiness; [2]Undergraduated student of Technology in Agribusiness of the Faculty of Technology of São José do Rio Preto - FATEC, Brasil (mariana_taglietto@hotmail.com); [3]FATEC, Brasil; [4]Oligos Biotecnologia

MC-17 **Insecticidal potential of new** ***Bacillus thuringiensis*** **and** ***Lysinibacillus sphaericus*** **strains against** ***Spodoptera frugiperda*** **(Lep. Noctuidae).** Maximiano Cassal, Gabriela Cristina Alles, Diouneia Lisiane Berlitz and Lidia Mariana Fiuza. UNISINOS, Laboratory of Microbiology and Toxicology, PPG in Biology, Av. Unisinos, 950 – CEP 93022-000, São Leopoldo, RS, Brazil

MC-18 **Toxicity of transgenic indica** ***Bt*****-rice (IRGA-424), expressing Cry1Aa toxin from** ***Bacillus thuringiensis*** **to** ***Spodoptera frugiperda*** **(Lepidoptera: Noctuidae), in laboratory.** Laura Massochin Nunes Pinto[1], Caroline Agriardi[1], Fernanda Pavani[1], Shana Wiest[1], Jaime Oliveira[2], Valmir Menezes[2], Athos Gadea[2], Maurício Fischer[2], Pascal Gantet[3], Emmanuel Guiderdoni[3] and Lidia Mariana Fiuza[1,2]. [1]UNISINOS, Laboratory of Microbiology and Toxicology. CEP 93001-970, São Leopoldo, RS/Brazil; [2]IRGA/EEA, Rice Experiment Station, CEP 94930-030, Cachoerinha, RS/Brazil. [3]CIRAD, Development and Plant Breeding, Team "Rice Adaptive Development", Av. Agropolis, 34398 Montpellier/France

MC-19 **New** ***B. thuringiensis*** **isolates with high toxic activity against Lepidopteran larvae in Mexico.** María Guadalupe Maldonado-Blanco[1], José Fernando Ornelas Pérez[1], Myriam Elías-Santos[1], Mónica Guadalupe Lozano-Contreras[2].[1]Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. Av. Pedro de Alba y Manuel L. Barragán s/n Ciudad Universitaria, C. P. 66450, A. P. 414 y 2790 San Nicolás de los Garza, Nuevo León, México. [2]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Mocochá, Km 25 Carretera Mérida-Motul, Mexico

MC-20 **Evaluation of native strains of** ***Isaria fumosorosea*** **(Wize) against** ***Anastrepha ludens*** **(Loew) (Diptera: Tephritidae) in Mexico.** Fátima Lizeth Gandarilla-Pacheco, Héctor Daniel Nava-González, Katiushka Arévalo-Niño, María Guadalupe Maldonado Blanco and Isela Quintero-Zapata. Instituto de Biotecnología, Facultad de Ciencias Biológicas. Universidad Autónoma de Nuevo León (UANL). 66450 San Nicolás de los Garza, N.L., Mexico

MC-21 **IMBICONT: Improved biological control for IPM in fruits and berries.** Italo Delalibera Jr.[1], Jørgen Eilenberg[2], Annette Bruun Jensen[2], Celeste D'Alessandro[1], Lene Sigsgaard[2] and Sílvia Helena Galvão de Miranda[3]. [1]Department of Entomology and Acarology, [3]Department of Economics, Business and Sociology, ESALQ, University of São Paulo; [2]Department of Agriculture and Ecology, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frb C., Denmark

MC-22 **Increasing food availability by reducing crop losses for smallholder farmers.** Theresa Corless, Rob Reeder and Steve Edgington. CABI UK-Centre, Bakeham Lane, Egham, Surrey TW20 9TY, UK

MC-23 **Improvement of the economic feasibility of baculovirus production processes in insect cell cultures by use of the effluent for the production of high-value added goods: application to the production of *Bacillus thuringiensis.*** Gabriela Analía Micheloud[1,2], Verónica Viviana Gioria[1,2], Gustavo Pérez[3] and Juan Daniel Claus[1,2]. [1]Laboratory of Virology, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, [2]Instituto de Agrobiotecnología del Litoral (IAL), CONICET/UNL, and [3]Department of Economy, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, (3000) Santa Fe, República Argentina

Poster Session 1 **Monday, 16:30 – 18:30**
Viruses **Room 3**

V-01 **Complete sequence and genomic analysis of the *Hyphantria cunea* granulovirus.** Jae Bang Choi, Won Il Heo, Sung Min Bae, Tae Young Shin, Jun Beom Lee, Yeon Ho Je[1], Byung Rae Jin[2] and Soo Dong Woo. Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea, [1]School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, Korea, [2]College of Natural Resources and Life Science, Dong-A University, Busan, Korea

V-02 **Occurrence and genetic variability of CpGV infecting *Cydia pomonella* at different geographical locations in Argentina.** Joel D. Arneodo[1], O. Marcelo Farinon[1], Ricardo Salvador[1], Karolin Eberle[2], Alicia Sciocco-Cap[1], Johannes Jehle[2] and Graciela Quintana[1]. [1]Instituto de Microbiología y Zoología Agrícola (IMYZA-CICVyA-INTA), Buenos Aires, Argentina. [2]Institute for Biological Control, Julius Kühn-Institute, Darmstadt, Germany

V-03 **Ocurrence and phylogenetic characterization of a baculovirus isolated from *Culex quinquefasciatus* in São Paulo State, Brazil**. Carlos José Pereira da Cunha de Araujo-Coutinho[1], Rafael Alves[1], Neil D. Sanscrainte[3], Andréa de Barros Pinto Viviani[2], Paulo Frugoli dos Santos[2], Polyana A. Vasconcelos-Medeiros de Souza[1], Isabel Maria Vicente Guedes de Carvalho-Mello[1] and James J. Becnel[3]. [1]Instituto Butantan, Laboratório de Imunologia Viral, Av. Vital Brazil nº 1500, 05503-900 São Paulo, SP, Brazil; [2]Superintendência de Controle de Endemias, Av. Pernambuco 1045, 11665-070 Caraguatatuba, SP, Brazil; [3]Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Florida, US

V-04 **Genetic diversity among isolates of *Autographa californica multiple nucleopolyhedrovirus.*** Robert L. Harrison[1], Holly J. R. Popham[2], Jonathan E. Breitenbach[2] and Daniel L. Rowley[1]. [1]Invasive Insect Biocontrol and Behavior Laboratory, Plant Sciences Institute, USDA Agricultural Research Service, 10300 Baltimore Avenue, Beltsville, Maryland 20705, USA, [2]Biological Control of Insects Research Laboratory, USDA Agricultural Research Service, 1503 S. Providence Road, Columbia, Missouri 65203, USA

V-05 **Complete sequence comparison between three genetically distinct *Bombyx mori* nucleopolyhedrovirus isolates in Korea.** Won Il Heo, Jae Bang Choi, Sung Min Bae, Tae Young Shin, Jun Beom Lee, Yeon Ho Je[1], Byung Rae Jin[2] and Soo Dong Woo. Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea; [1]School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, Korea; [2]College of Natural Resources and Life Science, Dong-A University, Busan, Korea

V-06 **Lack of stability of the infectivity of budded virus of *Anticarsia gemmatalis multiple nucleopolyhedrovirus* in serum-free medium supplemented with lipid microemulsions.** Ignacio Eberhardt[1,2], Verónica Viviana Gioria[1,2], Gabriela Analía Micheloud[1,2] and Juan Daniel Claus[1,2]. [1]Laboratory of Virology, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, and [2]Instituto de Agrobiotecnología del Litoral (IAL), CONICET/UNL, (3000) Santa Fe, República Argentina

V-07 **Sequential passage of the Nucleopolyhedrovirus of *Anagrapha falcifera* (AfMNPV) in larvae of *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae).** Fabiane Cunha[12], Flavio Moscardi[12], Maria E.B. Castro[3], Moema T. Castro[3], Zilda M.A. Ribeiro[3], Ângela Falleiros[1], Sheila M. Levy[1], Mauricio L. Moscardi[1], Talita M. Alexandre[1] and Daniel R.Sosa-Gomez[4]. [1]Agronomy Department, Universidade Estadual de Londrina, 86051-970 - Londrina, PR; [2]Agronomy Department, Universidade do Oeste Paulista, 19050-920 – Presidente Prudente, SP; [3]Embrapa Recursos Genéticos e Biotecnologia, 70770-917 – Brasilia, DF; [4]Embrapa Soja, 86001-970 – Londrina, PR, Brazil

V-08 **STU** **Analysis of recombinant protein expression in *Anticarsia gemmatalis* larvae infected with recombinant AgMNPV baculoviruses containing the firefly luciferase gene under the control of early and late promoters.** Fabricio da Silva Morgado, Daniel M. P. Ardisson-Araújo, Daniele Vitoriana Freitas, Raíssa Allan Santos Domingues and Bergmann Morais Ribeiro. Laboratório de Microscopia Eletrônica e Virologia, Departamento de Biologia Celular, Instituto de Ciências Biologicas, Universidade de Brasília

V-09 **Prediction and detection of a viral microRNA in AgMNPV infected High Five cells.** Carina Reyes[1], M. Leticia Ferrelli[1], M. Laura García[1], P. Daniel Ghiringhelli[2] and Víctor Romanowski[1]. [1]Instituto de Biotecnología y Biología Molecular, Universidad Nacional de La Plata, CONICET, Argentina; [2]Laboratorio de Ingeniería Genética y Biología Celular y Molecular, Area Virosis de Insectos, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Argentina

V-10 **STU** **Proteomics of the *Anticarsia gemmatalis* multiple nucleopolyhedrovirus budded viruses.** Diego Luis Mengual Gómez, Mariano Nicolás Belaich and Pablo Daniel Ghiringhelli. LIGBCM-AVI, Laboratorio de Ingeniería Genética y Biología Celular y Molecular- Área Virosis de Insectos, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, Bernal, Buenos Aires, Argentina

V-11 **STU** **Evaluation of AgMNPV replication based on HRs sequences.** Solange Ana Belen Miele, Mariano Nicolas Belaich and Pablo Daniel Ghiringhelli. LIGBCM-AVI, Laboratorio de Ingeniería Genética y Biología Celular y Molecular- Área Virosis de Insectos; Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, Bernal, Buenos Aires, Argentina

V-12 **Susceptibility evaluation of six insect cell lines to *Spodoptera frugiperda multiple nucleopolyhedrovirus.*** Jorge O. Mateus[1], William Sihler[1], ZiIda Maria A. Ribeiro[1], Fernando H. Valicente[2] and Marlinda L. Souza[1]. [1]Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Av. W5 Norte final, Brasília, DF, Brasil, CEP 70.770-900, [2]Embrapa Milho e Sorgo, Rod MG 424 Km 65 Sete Lagoas, MG, Brasil, CEP 35701-970

V-13 **Ultrastructural analysis of six *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV) Many Polyhedra variants.** Camilla R. Teixeira, William Sihler, Rosana Falcão, Bergmann M. Ribeiro and Marlinda L. Souza. [1]Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Av. W5 Norte final, Brasília, DF, Brasil, CEP 70.770-900, [2]Universidade de Brasília, Departamento de Biologia Celular, Prédio K, Brasília, DF, Brasil, 70910-900

V-14 **Laboratory and field populations of *Spodoptera exigua* are naturally infected by multiple viruses.** Cristina Virto[1], David Navarro[1,3], Mª del Mar Tellez[3], Salvador Herrero[4], Trevor Williams[5], Rosa Murillo[1,2] and Primitivo Caballero[1,2]. [1]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Ctra. de Mutilva s/n 31192, Mutilva Baja, Spain; [2]Departamento Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain; [3]IFAPA, La Mojonera, 04745, Almería, Spain; [4]Departamento de Genética, Universitat de Valencia, Spain; [5]Instituto de Ecología AC, Xalapa 91070, Mexico

V-15 **STU** **The role of *Spodoptera exigua* multiple nucleopolyhedrovirus genes *se76* and *se28* on viral pathogenicity.** Amaya Serrano[1], Gorben Pijlman[2], Monique van Oers[2], Trevor Williams[3], Delia Muñoz[4] and Primitivo Caballero[1,4]. [1]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, 31192 Mutilva Baja, Navarra, Spain; [2] Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [3] Instituto de Ecología AC, Apartado Postal 63, Xalapa, Veracruz 91070, Mexico; [4] Departamento de Producción Agraria, Universidad Pública de Navarra, 31006 Pamplona, Spain

V-16 **Sex-specific variation in vertical transmission of SeMNPV.** Carlos Andrés Zarate, Cristina Virto[1], Rosa Murillo[1,2], Trevor Williams[3] and Primitivo Caballero[1,2]. [1] Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Ctra. de Mutilva s/n 31192, Mutilva Baja, Spain; [2] Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain; [3] Instituto de Ecología AC, Xalapa 91070, Mexico

V-17 **Biological comparison of four nucleopolyhedrovirus isolates of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae).** Fidencio Álvarez-Antúnez[1,2], Ovidio Díaz-Gómez[2], Norma Zamora-Avilés[1], Marcelo Berretta[3], Alicia Sciocco-Cap[3], Samuel Pineda-Guillermo[1], José Isaac Figueroa de la Rosa[1] and Ana Mabel Martínez-Castillo[1]. [1]Instituto de Investigaciones Agropecuarias y Forestales, Michoacán, Mexico. [2]Universidad Autónoma de San Luis Potosí. Facultad de Agronomía, San Luis Potosí, Mexico. [3]Instituto de Microbiología y Zoología Agrícola (IMYZA-CICVyA), INTA Castelar, Argentina

V-18 **Combined effects of azadirachtin and a nucleopolyhedrovirus (SfMNPV) on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae.** Norma Zamora Avilés[1], Jorge Alonso Vargas-Leandro[2], Samuel Pineda-Guillermo[1], José Isaac Figueroa de la Rosa[1], Juan Manuel Chavarrieta[1] and Ana Mabel Martínez-Castillo[1]. [1]Instituto de Investigaciones Agropecuarias y Forestales, Michoacán, Mexico. [2]Instituto Tecnológico de Costa Rica, Cartago, Costa Rica

V-19 **STU** **Feeding, growth and toxicity evaluation of microbial insecticides *Spodoptera* Nucleopolyhedrovirus (Splt NPV) against *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae).** Thiyagarajan Nataraj, Kadarkarai Murugan and Pari Madhiyazhagan. Division of Entomology, Department of Zoology, Bharathiar University, Coimbatore, India

Divisions Business Meetings

Evening Session 1 **Bacteria Division Business Meeting** Organizer: Juan Luis Jurat Fuentes	**Monday, 18:45 – 20:00** **Auditorium 3**
Evening Session 2 **Fungi Division Business Meeting** Organizer: Helen Roy	**Monday, 18:45 – 20:00** **Auditorium 4**
Evening Session 3 **Microsporidia Division Meeting** Organizer: Dörte Goertz	**Monday, 18:45 – 20:00** **Room 1**
Evening Session 4 **Nematodes Division Business Meeting** Organizer: Ed Lewis	**Monday, 18:45 – 20:00** **Room 2**
Evening Session 5 **Virus Division Business Meeting** Organizer: Monique Van Oers	**Monday, 18:45 – 20:00** **Auditorium 2**

TUESDAY – August 7th

Registration	**Tuesday, 08:00 – 12:00**
Morning Session 1 **Symposium II -** Microsporidia Division	**Tuesday, 08:00 – 10:00** **Room 2**

Microsporidia from South America

Organizers: Carlos Lange and Dörte Goertz

08:00 **35** ***Edhazardia aedis*, a microsporidian pathogen of *Aedes aegypti*: Possibilities and challenges for classical biocontrol in South America.** James J. Becnel. Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL 32608, USA

08:30 **36** **Native and alien microsporidia in Argentine grasshoppers.** Carlos E. Lange. Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CIC – UNLP – CCT La Plata CONICET, Argentina

09:00 **37** **Microsporidian isolates from mosquitoes of Argentina.** María Victoria Micieli[1], Theodore G. Andreadis[2], Charles R. Vossbrinck[2], James J. Becnel[3]and Juan José García[1]. [1]Centro de Estudios Parasitológicos y de Vectores, CEPAVE (CONICET-CCT La Plata-UNLP)-, calle 2 N° 584, (1900) La Plata, Buenos Aires, Argentina. [2]Center for Vector Biology & Zoonotic Diseases, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, USA. [3]USDA, ARS, CMAVE 1600 S.W. 23rd Drive Gainesville, FL 32608, USA

09:30 **38 Microsporidia from honey bees and bumble bees in southern South America.** Santiago Plischuk[1], Mariano Higes[2] and Carlos E. Lange[1]. [1]Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CCT La Plata-CONICET –CICPBA –UNLP, La Plata, Argentina; [2]Laboratorio de Patología, Centro Apícola de Marchamalo, Junta de Comunidades de Castilla-La Mancha, Spain

Morning Session 2 **Tuesday, 08:00 – 10:00**
Symposium III - Fungi Division **Auditorium 2**

Host Immune Response to Fungal Pathogens

Organizers: Joanna Fisher and Ann Hajek

08:00 **39 Metapleural gland secretion, an extra anti-fungal cuticular immune system of leaf-cutting ants.** Sze Huei Yek[1], David R. Nash[1], Annette B. Jensen[2] and Jacobus J. Boomsma[1]. [1]Centre for Social Evolution, Department of Biology, University of Copenhagen, 7 Universitetsparken 15, 2100 Copenhagen, Denmark; [2]Centre for Social Evolution,Department of Agriculture and Ecology, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frb C., Denmark

08:15 **40 Avoidance of insect pathogenic fungi by predatory insects.** [1]Nicolai Meyling, [2] Helen Hesketh and [2]Helen Roy. [1]Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Denmark. [2]NERC Centre for Ecology & Hydrology, Crowmarsh Gifford, Oxfordshire, OX10 8EF, UK

08:30 **41 Fungal pathogens and temperature stress affect gene expression patterns in bees.** Rosalind James[1] and Junhuan Xu[2]. [1]USDA-ARS Pollinating Insects Research Unit, Logan, UT; [2]Dept. Biology, Utah State University, Logan, UT

09:00 **42 An antifungal defense strategy in termites and woodroaches.** Mark S. Bulmer. Towson University, Townson, MD, USA

09:30 **43 Sensitivity of behavior to pathogen-related odor in the termite *Coptotermes formosanus.*** Aya Yanagawa[1], Nao Fujiwara-Tsujii[2], Toshiharu Akino[3], Tsuyoshi Yoshimura[1] and Susumu Shimizu[4]. [1]Research Institute for Sustainable Humanosphere, Kyoto University, Gokashou, Uji, 611-0011, Japan; [2]National Institute of Agrobiological Science, Ohwashi, Tsukuba, 305-0851, Japan; [3]Department of Biology, Kyoto Institute of Technology, Matsugasaki, Kyoto, 606-8585, Japan; [4]Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka, 812-8581, Japan

Morning Session 3 **Tuesday, 08:00 – 10:00**
Contributed Papers **Auditorium 4**

Bacteria 2

Chairs: Juan Ferre and Marianne Carey

08:00 **44 Susceptibility of *Aedes aegypti* populations to *Bacillus thuringiensis israelensis* with different status of organophosphate resistance.** Maria Alice V. Melo-Santos, Elisama E. Helvecio, Ana Paula A. P. Araújo, Diego D. F. A. Diniz, Andréa N. Souza, Rosineide R. A. Barros, Claúdia M. F. Oliveira, Constância F. J. Ayres and Maria Helena N. L. Silva-Filha. Department of Entomology, Centro de Pesquisas Aggeu Magalhães-FIOCRUZ, Recife- PE, 50670-420 Brazil

08:15 **45 Novel mutations associated to *Bacillus sphaericus* resistance are identified in a polymorphic region of the *Culex quinquefasciatus cqm1* gene.** Karlos D. M. Chalegre[1], Tatiany P. Romão[1], Daniella A. Tavares[1], Eloína M. Santos[1], Lígia M. Ferreira[1], Cláudia M. F. de Oliveira[1], Osvaldo P. de-Melo-Neto[2] and Maria Helena N. L. Silva-Filha[1]. [1]Department of Entomology and [2]Department of Microbiology, Centro de Pesquisas Aggeu Magalhães-FIOCRUZ, Recife- PE, 50670-420 Brazil

08:30 **46 Resistance mechanisms of *Galleria mellonella* (Lepidoptera, Pyralidae) larvae under selection by bacteria *Bacillus thuringiensis.*** Ivan Dubovskiy[1], Ekaterina Grizanova[1], Irina Slepneva[2]and Viktor Glupov[1]. [1]Institute of Systematics and Ecology of Animals, Siberian Branch Russian Academy of Sciences, Novosibirsk, Russia; [2] Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

08:45 **47 STU Potential of resistance to *Bacillus thuringiensis* in a greenhouse population of *Ostrinia nubilalis* (Hübner).** Cristina M. Crava, Yolanda Bel, Juan Ferréand Baltasar Escriche. Department of Genetics, University of Valencia. 46100 Burjassot, Valencia, Spain

09:00 **48 Specific binding of radiolabeled Cry1Fa toxin from *Bacillus thuringiensis* in susceptible lepidopteran species and resistant diamondback moth.** Patricia Hernández-Martínez[1], Carmen Sara Hernández-Rodríguez[1], Vidisha Krishnan[2], Neil Crickmore[2], Jeroen Van Rie[3], Baltasar Escriche[1] and Juan Ferré[1]. [1]Departamento de Genética, Facultad de CC. Biológicas, Universidad de Valencia, Spain; [2]School of Life Sciences, University of Sussex, Brighton, GB; [3]Bayer CropScience, Ghent, Belgium

09:15 **49 Mechanism of field-evolved resistance to transgenic Bt corn in *Spodoptera frugiperda.*** Siva R. K. Jakka, Liang Gong, and Juan Luis Jurat-Fuentes. Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA

09:30 **50 STU Fitness costs in *Spodoptera frugiperda* with field-evolved resistance to Bt corn.** S.R.K. Jakka, V.R. Knight and J.L. Jurat-Fuentes. Department of Entomology and Plant Pathology, University of Tennessee, Knoxville (TN) 37996, USA

09:45 **51 Resistance of western corn rootworm to Bt maize.** Aaron J. Gassmann, Jennifer L. Petzold-Maxwell, Eric H. Clifton, Mike W. Dunbar, Amanda M. Hoffmann, David A. Ingber and Ryan S. Keweshan. Department of Entomology, Iowa State University, Ames, IA USA

Morning Session 4 **Tuesday, 08:00 – 10:00**
Contributed Papers **Auditorium 3**

Viruses 1

Biocontrol and Biotechnology
Chairs: Gabriel A. Visnovsky and Lorena Passarelli

08:00 **52 Effects of adjuvants on pathogenicity of *Plutella xylostella* granulovirus (*PlxyGV*) on diamondback moth, (L.) (Lepidoptera: Plutellidae).** Ahmad Dezianian[1], Ahmad Said Sajap[2], Wei Hong Lau[3], Dzolkhifli Omar[3], Hussan Abdol Kadir[4], Mohamed Rozi[2] and Mohamed Rani Mat Yusoh[4]. [1]Department of Plant Protection, Shahrood (Semnan) Agricultural Research Centre, Bastam highway, P.O. Box; 36155-313,Shahrood, Iran. [2]Department of Forest Management, Faculty of Forestry, University Putra Malaysia, 43400 UPM Serdang, Selangor, DE, Malaysia. [3]Department of Plant Protection, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, DE, Malaysia [4]Malaysia Agricultural Research and Development Institute (MARDI), Serdang, Selangor, DE, Malaysia

08:15 **53 STU Lethal concentration dependent interaction of a *Agrotis segetum* nucleopolyhedrovirus and granulovirus in mixed infections.** Jörg Thomas Wennmann, Gianpiero Gueli Alletti and Johannes Alois Jehle. Institute for Biological Control, Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Darmstadt, Germany

08:30 **54 A tarantula toxin causes early cell death during in vitro insect cell infection by a recombinant baculovirus.** Daniel M. P. Ardisson-Araújo[1], Fabrício S. Morgado[1], Roberto F.Teixeira[1], Elizabeth N. F. Schwartz[1], Gerardo Corzo[2] and Bergmann M. Ribeiro[1]. [1] Department of Cell Biology, Institute of Biological Science, University of Brasília, Brazil.[2] Institute of Biotechnology, UNAM, Mexico

08:45 **55 STU *In vivo* monitoring of protein expression in insect cells using recombinant AgMNPV baculoviruses.** Fabricio da Silva Morgado, Daniel M. P. Ardisson-Araújo and Bergmann Morais Ribeiro. Laboratório de Microscopia Eletrônica e Virologia, Departamento de Biologia Celular, Instituto de Ciências Biologicas, Universidade de Brasília. Brazil

09:00 **56 STU Host impact on *Anticarsia gemmatalis* multiple nucleopolyhedrovirus production.** Diego Luis Mengual Gómez[1], Mariano Nicolás Belaich[1], Alicia Sciocco-Cap[2] and Pablo Daniel Ghiringhelli[1]. [1]LIGBCM-AVI. Laboratorio de Ingeniería Genética y Biología Celular y Molecular- Área Virosis de Insectos), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes (Roque Sáenz Peña 352, Bernal, Buenos Aires, Argentina). [2]IMyZA-CCVyA/INTA, Las Cabañas y los Reseros s/n, Hurlingham, Argentina

09:15 **57 Growth of the UFL-AG-286 cell line and replication of the *Anticarsia gemmatalis multiple nucleopolyhedrovirus* in a new medium free of animal protein hydrolysates.** María Alejandra Baqué[1], Verónica Viviana Gioria[1,2], Gabriela Analía Micheloud[1,2] and Juan Daniel Claus[1,2]. [1]Laboratory of Virology, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, and [2]Instituto de Agrobiotecnología del Litoral (IAL), CONICET/UNL, (3000) Santa Fe, República Argentina

09:30 **58 Towards a feasible process for the large scale production of Oryctes virus in DSIR-HA-1179 insect cell cultures.** Gabriel Alberto Visnovsky[1], Juan Daniel Claus[2] and Charlotte Pushparajan[1]. [1]Department of Chemical and Process Engineering, University of Canterbury, New Zealand and [2]Lab. Virología, Facultad de Bioquimica y Ciencias Biologicas, Universidad Nacional del Litoral, Santa Fe, Argentina

09:45 **59 Baculovirus deleted for *chitinase*, *cathepsin* and *p10* genes improves rAAV8 vector integrity and infectiosity.** Lionel Galibert[1], Christel Rivière[1], Bérangère Langlet[1], Marjorie Boutin Fontaine[1], David Cohen[2], Monique Van Oers[2]and Otto-Wilhelm Merten[1]. [1]Généthon, 1 bis rue de l'Internationale, 91002 Evry, France. [2]University of Wageningen, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

10:00 – 10:25 **BREAK**

Mid-Morning Session 1 **Tuesday, 10:30 – 12.30**
Symposium IV - Diseases of Beneficial Invertebrates Division **Auditorium 3**

Global bee health and specific issues in Latin America

Organizers: Elke Genersch and Adriana Alippi

10:30 **60 Colony collapse occurrence in Africanized honey bees in Brazil.** D. Message[1], I.C. Silva[2], Z.L.P. Simões[3], E.W.Teixeira[4] and D. De Jong[5]. [1]Retired Professor from Departamento de Biologia Animal/UFV, 36570-000 Viçosa/MG/Brasil; [2]FFCLRP-USP - Depto Biologia, 14049-900 Ribeirão Preto/SP,Brasil; [3]FFCLRP-USP - Depto Biologia, 14049-900 Ribeirão Preto/SP,Brasil; [4]APTA/DDD/Polo Regional – Caixa Postal 07, 12422-970, Pindamonhangaba/SP, Brasil; [5]Depto Genética-FMRP/USP, Ribeirão Preto,SP,Brasil

11:00 **61 Status of pathogens and other potential enemies of native bumblebees in Argentina** Matías Daniel Maggi[1], Santiago Plischuk[2], Pablo Revainera[1], Mariano Lucía[3] and Alberto Abrahamovich[3]. [1]Laboratorio de Artrópodos, Facultad de Ciencias Exactas y Naturales. UNMDP-CONICET; [2] Centro de Estudios Parasitológicos y de Vectores (CEPAVE)-CONICET; [3]Laboratorio de Apidología, División Entomología, Museo de La Plata (MLP), Univ. Nac. de La Plata-CONICET, Argentina

11:30 **62 Epidemiology of Tetracycline resistant strains of *Paenibacillus larvae,* the cause of American Foulbrood, in the Americas** Adriana M. Alippi -CIDEFI- Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, calle 60 y 119 S/N (1900), Argentina

12:00 **63 Molecular pathogenesis of American Foulbrood, a globally occurring epizootic of honey bees.** Elke Genersch, Anne Fünfhaus, Eva Garcia-Gonzalez, Gillian Hertlein and Lena Poppinga. Institute for Bee Research, Friedrich-Engels-Str. 32, D-16540 Hohen Neuendorf, Germany

Mid-Morning Session 2 **Tuesday, 10:00 – 12:30**
Symposium V - Nematodes Division **Room 1**

EPN Discovery and Implementation in Latin America: Current Research and Future Directions

Organizer: Patricia Stock

10:30 **64 Current status on the discovery and implementation of EPN in Brazil and Argentina.** S. Patricia Stock. Department of Entomology, The University of Arizona, Tucson, AZ, USA

11:00 **65 Entomopathogenic nematodes in Venezuela: A short history with a promising future.** Ernesto San-Blas. Laboratorio de Protección Vegetal, Centro de Estudios Botánicos y Agroforestales, Instituto Venezolano de Investigaciones Científicas, Maracaibo, Venezuela

11:30 **66 Development and use of entomopathogenic nematodes in Cuba.** Mayra G. Rodríguez-Hernández [1], Roberto Enrique[1], Esteban González[1], Lucila Gómez[1], Dainé Hernández-Ochandía[1], Lidia López[1], Mario Hernández[2], Miguel A. Hernández[1], Yusney Borrero[2], Luisa Díz-Viruliche[3] and Belkis Peteira[1]. [1]Centro Nacional de Sanidad Agropecuaria (CENSA), Apartado 10, San José de las Lajas, Mayabeque, Cuba. [2]Centro Nacional de Referencia Fitosanitaria para la Montaña (CNRFM), Buey Arriba, Granma. Cuba. [3]Universidad Agraria de La Habana, San José de las Lajas, Mayabeque, Cuba

12:00 **67 Perspective and research of Entomopathogenic Nematodes in Chile.** Andrés France. INIA Quilamapu, Casilla 426, Chillán, Chile

Mid-Morning Session 3 **Tuesday, 10:30-12:30**
Contributed Papers **Auditorium 4**

Fungi 2

Chairs: Nina Jenkins and Drauzio Rangel

10:30 **68 Proteomic analysis of native strains of *Beauveria bassiana* and *Metarhizium anisopliae* and their toxicity against soybean weevil.** Cipriano García-Gutiérrez, J Manuel Mancillas-Paredes and Sergio Medina-Godoy. CIIDIR-COFAA IPN Sinaloa. Department of Biotechnology. Blvd. Juan de Dios Batiz Paredes No. 250 AP. 280 Guasave, Sinaloa, Mexico, CP 8110

10:45 **69 A 1,4-benzoquinone reductase of the entomopathogenic fungus *Beauveria bassiana* is involved in the degradation of *Tribolium castaneum* defensive secretions.** Nicolás Pedrini[1], Yanhua Fan[2,3], M. Patricia Juárez[1] and Nemat O. Keyhani[3]. [1]Instituto de Investigaciones Bioquímicas de La Plata, Facultad de Ciencias Médicas (UNLP), Calles 60 y 120, La Plata, Argentina; [2]Biotechnology Research Center, Southwest University, Beibei, Chongqing, China; [3]Dept. of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611

11:00 **70 Characterization of a hydrophobin gene promoter for efficient gene expression in *Beauveria bassiana*** Zhengliang Wang and Ming-guang FengInstitute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou 310058, P.R. China

11:15 **71 A Class III histidine kinase gene (*BbHK1*) regulates conidiation in entomopathogenic fungi *Beauveria bassiana.*** Lei Qiu and Ming-guang Feng. Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou 310058, P.R. China

11:30 **72 The *Beauveria bassiana* gene *Bbpmr1* is important for cation homeostasis, conidiation, multi-stress tolerance and virulence.** Jie Wang and Ming-Guang Feng. Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou 310058, P.R. China

11:45 **73 The cell wall integrity in entomopathogen *Beauveria bassiana* depends on mitogen-activated protein kinase kinase signaling pathway** Ying Chen and Ming-guang Feng. Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou 310058, P.R. China

Mid-Morning Session 4 **Tuesday, 10:30-12:30**
Contributed Papers **Room 2**

Microbial Control 1

Chairs: Jorge Ibarra and Iñigo Ruiz de Escudero

10:30 **74 STU Toxicity of Cry1 and Vip3A proteins to *Diatraea saccharalis* (F, 1794) (Lepidoptera: Pyralidae) and binding to brush border membrane vesicles.** Camila C. Davolos[1,2], Patricia Hernández-Martínez[2], Cristina M. Crava[2], Juan Ferré[2], Janete A. Desidério[1], Manoel Victor F. Lemos[1] and Baltasar Escriche[2]. [1]Department of Applied Biology, São Paulo State University, Jaboticabal (São Paulo), Brazil; [2]Department of Genetics, University of Valencia, 46100-Burjassot (Valencia), Spain

10:45 **75 STU Cool Caterpillars: Low temperature biological control of a climbing cutworm.** T. Scott Johnson[1], Tom Lowery[2], Joan Cossentine[2] and Jenny Cory[1]. [1]Simon Fraser University, Burnaby BC Canada; [2]Agriculture and Agri-Food Canada, Summerland BC Canada

11:00 **76 STU Insect-specific sodium ion pump targeting μ-Agatoxin IV peptide inhibits *Trichoderma asperellum* conidiation** Babak Pakdaman Sardrood[1], Ebrahim Mohammadi Goltapeh[1], Joanna Kruszewska[2], Bahram Mohammad Soltani[3], Sebastina Pilzyk[2], Monika Komon-Zelazowska[4], Irina Druzhinina[4], Magsood Pajhoohandeh[5], Sabrina Sarrocco[6], Giovani Vannacci[6], Christian Peter Kubicek[4], and Holger Bruno Deising[7]. 1. Department of Plant Pathology, Agricultural Faculty, Tarbiat Modares University, Tehran, Iran; 2. Laboratory for Fungal Glycobiology, Department of Genetics, Institute of Biochemistry and Biophysics, Warsaw, Poland; 3. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran; 4. Institute of Chemical Engineering, Faculty of Technical Cemistry, Vienna University of Technology, Vienna, Austria; 5. Group of Biotechnology, Faculty of Agriculture, Azarbaijan Tarbiat Moallem University, Tabriz, Iran; 6. Department of Tree Science, Entomology and Plant Pathology, Faculty of Agriculture, University of Pisa, Pisa, Italy; 7. Work Group of Plant Sciences, Institute of Agricultural and Nutritional Sciences, Faculty of Natural Sciences III, Martin-Luther-University, Halle-Wittenberg, Halle, Germany

11:15 **77 STU Hybrid approach to the control of greenhouse whitefly in Australia.** Jennifer E Spinner[1], Bree AL Wilson[1], Ben J Stodart[1], Caroline Hauxwell[2] and Gavin J Ash[1]. [1]EH Graham Centre for Agricultural Innovation (Charles Sturt University and Industry & Investment NSW), Boorooma Street, Wagga Wagga, NSW 2678; [2]Queensland University of Technology, George St, Brisbane QLD

11:30 **78 Biopesticide potential of organisms from ecological extremes.** Steve Edgington[1], Emma Thompson[1], Dave Moore[1], Kevin Hughes[2], Andrés France[3] and Paul Bridge[1]. [1] CABI UK-Centre, Bakeham Lane, Egham, Surrey TW20 9TY, UK; [2]British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET; [3] Instituto de Investigaciones Agropecuarias (INIA), Avenida Vicente Méndez, Casilla 426, Chillán, Chile

11:45 **79 *Paecilomyces lilacinus:* possible candidate to control the leaf-cutter ant *Acromyrmex lundii?*** Daniela Goffré and Patricia J. Folgarait. Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes. Roque Saenz Peña 352, Bernal, Buenos Aires, Argentina

12:00 **80 Can a leaf-cutter *Paecilomyces lilacinus* strain be used to control red fire ants?** Patricia J. Folgarait, Alejandra Habarta, Daniela Goffré and Lawrence E. Gilbert[1]. Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Buenos Aires, Argentina. [1]University of Texas-Austin and Brackenridge Field laboratory, Texas, USA

Mid-Morning Session 5 **Tuesday, 10:30-12:15**
Contributed Papers **Auditorium 2**

Viruses 2

Genomes and Transcriptomes
Chairs: Robert Harrison and Elisabeth Herniou

10:30 **81 Genome sequence and organization of a baculovirus isolated from *Perigonia lusca* (Lepidoptera: Sphingidae).** Fernando L. Melo[1], Daniel M. P. Ardisson-Araújo[1], Fabricio S. Morgado[1], Daniele V. Freitas[1], Miguel Andrade[1], Daniel R. Sosa-Gomez[2], Bergmann M. Ribeiro[1].[1] Department of Cell Biology, Institute of Biological Science, University of Brasília, Brazil.[2] Centro Nacional de Pesquisa da Soja, EMBRAPA - Londrina, PR, Brazil

10:45 **82 STU Ultra-deep sequencing of AcMNPV and comparison to original genome sequencing.** Aurélien Chateigner[1], Davy Jiolle[1], Carole Labrousse[1], Annie Bézier[1] and Elisabeth Herniou[1]. [1]Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 6035, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Avenue Monge - Parc Grandmont 37200 Tours France

11:00 **83 STU Transcriptome analysis of the *Cydia pomonella* granulovirus.** Diana Schneider, Karolin Elisabeth Eberle and Johannes Alois Jehle. Julius Kühn-Institut, Institute for Biological Control, Heinrichstraße 243, 64287 Darmstadt, Germany

11:15 **84 STU Ac53, ac78, ac101 and ac103 are newly discovered core genes in the family *Baculoviridae.*** Matias Javier Garavaglia[1], Solange Ana Belén Miele[1], Iserte Javier Alonso[2], Belaich Mariano Nicolas[1] and Ghiringhelli Pablo Daniel[1].[1] LIGBCM-AVI, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Saenz Peña 352, Bernal, Argentina. [2]LIGBCM-AVEZ, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Saenz Peña 352, Bernal, Argentina

11:30 **85 Nucleopolyhedrosis causing virus from the crane fly *Tipula oleracea.*** Annie Bézier[1], Darren Obbard[2], Julien Thézé[1] and Elisabeth A. Herniou[1]. [1]Insect Biology Research Institute, CNRS UMR 7261, University François Rabelais, 37200 Tours, France; [2] Institute of Evolutionary Biology**,** School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom

11:45 **86 STU Gene acquisition convergence drives adaptation in distant insect viruses.** Julien Thézé[1], Julie Gallais[1], Jun Takatsuka[2], Madoka Nakai[3], Elisabeth A. Herniou[1]. [1]Insect Biology Research Institute, CNRS UMR-7261, University François Rabelais, 37200 Tours, France. [2]Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba 305-8687, Japan. [3]Department of Applied Biological Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan

12:00 **87 STU Construction of an *Adoxophyes honmai* nucleopolyhedrovirus bacmid system to elucidate genes related to viral killing speed.** Yasumasa Saito, Yasuhisa Kunimi and Madoka Nakai. Graduate School of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu city, Tokyo 183-8509, Japan

13:00 Optional Excursion (Delta-Tigre; light lunch included). Buses leave from the Main Entrance of the UCA Convention Center.

18:30 – 21:30 BBQ (For delegates who do not participate in the excursion, buses will depart at 17:45 h from the Main Entrance of the UCA Convention Center)

WEDNESDAY – August 8th

Registration **Wednesday, 08:00-12:00**

Morning Session 1 **Wednesday, 08:00-10:00**
Symposium VI - Viruses and Fungi Divisions **Auditorium 2**

Pathogen induced host behaviour - clues for mechanisms

Organizers: Monique van Oers and Nicolai Vitt Meyling

08:00 **88 A behaviour-manipulating virus in a parasitoid wasp: genomics and transcriptomics insights.** Julien Varaldi, David Lepetit and Marie-Christine Carpentier. Laboratory of Biometry and Evolutionary Biology – UMR CNRS 5558. University Lyon 1. France

08:40 **89 Behavioural changes induced to hosts by Entomopthoralean fungi: mechanisms and evolutionary traits.** Jørgen Eilenberg, Joanna Małagocka and Annette Bruun Jensen. Department of Agriculture and Ecology, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frb C., Denmark

09.20 **90 Walking with insects: Molecular mechanisms behind parasitic manipulation of invertebrate host behaviour.** Vera I.D. Ros, Stineke van Houte and Monique M. van Oers. Laboratory of Virology, Wageningen University, The Netherlands

Morning Session 2 **Wednesday, 08:00 -10:00**
Symposium VII - Nematodes and Bacteria Divisions **Auditorium 4**

Beyond Agriculture:
Nematodes and Bacteria Applications in other Science Disciplines.

Organizers: Glen Stevens and Arne Peters

08:00 **91 *Photorhabdus* and *Xenorhabdus*: A drug discovery goldmine.** Nick R. Waterfield[1] and Helge B. Bode[2] (and the GAMEXP consortium). [1]Department of Biology and Biochemistry, University of Bath, BA2 7AY, UK; [2]Merck Stiftungsprofessur Molekulare Biotechnologie, Institut für Molekulare Biowissenschaften, Goethe Universität Frankfurt, Germany

08:30 **92 Endotoxin plasmids of *Bacillus thuringiensis*: from simple to complex genetic symbionts.** Brian A. Federici. Department of Entomology and Interdepartmental Graduate Program in Cell, Molecular and Developmental Biology, University of California, Riverside, USA

09:00 **93 Using nematodes to teach behavior: do worms and zebras really do the same things?** Edwin Lewis. Department of Nematology, University of California, Davis CA 95616, USA

09:30 **94 Entomopathogenic nematodes in the undergrad biology classroom: lessons in critical thinking.** Glen N. Stevens. School of Natural Sciences and Mathematics. Ferrum College, VA 24088, USA

10:00 – 10:25 **BREAK** **Setting up Poster Session 2 (Room 3)**

Mid-Morning Session 1 **Wednesday, 10:30-12:15**
Contributed Papers **Auditorium 4**

Bacteria 3

Chairs: Baltasar Escriche and Juan Luis Jurat-Fuentes

10:30 **95 Diversity and potential genomics mobility of the genetic determinants of cereulide, the *Bacillus cereus* emetic toxin.** Xiaomin Hu[1], Lingling Yang[1], Jacques Mahillon[2] and Zhiming Yuan[1]. [1]Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China; [2]Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, Louvain-la-Neuve, Belgium

10:45 **96 The 54-kDa protein of *Bacillus thuringiensis* subsp. *israelensis* required for parasporal body stability binds to individual endotoxin inclusions during their development.** Mercedes Diaz-Mendoza[1], Dennis K. Bideshi[1,2] and Brian A. Federici[1]. [1]Department of Entomology, University of California, Riverside, Riverside California 92521, and [2]California Baptist University, Riverside, California 92504, USA

11:00 **97 New mechanisms for "host iron" acquisition in *Bacillus cereus* and *B. thuringiensis*.** Diego Segond[1], Elise Abi khalil[1], Christophe Buisson, [1] Fadi Bou Abdallah ,[2] Mireille Kallassy, [3] Didier Lereclus and Christina Nielsen-LeRoux[1]. [1]INRA ,UMR 1319 Micalis, La Minière, 78650 Guyancourt cedex, France; [2]Department of Chemistry, SUNY, Potsdam, NY 13676, USA; [3]Laboratory of Biotechnology, Saint-Joseph University, Beyrouth, Lebanon

11:15 **98 Interactions of five Cry toxins with larval midgut binding sites of *Ostrinia furnacalis* (Guenée).** Xu Yang[1,2], Ning Li[1], Zhenying Wang[1], Qunfang Yang[2] and Kanglai He[1]. [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; [2] Sichuan Agricultural University, Ya'an, 625014, China

11:30 **99 *Bacillus thuringiensis* Cry34Ab1/Cry35Ab1 binding sites on *Diabrotica virgifera virgifera* LeConte midgut membranes are distinct from binding sites for Cry3Aa, Cry3Ba, Cry6Aa and Cry8Ba.** Huarong Li, Monica Olson, Gaofeng Lin, Tim Hey and Kenneth E. Narva. Dow AgroSciences LLC. 9330 Zionsville Road, Indianapolis, Indiana 46268, USA

11:45 **100 Aminopeptidases function as Cry11A toxin binding proteins in *Aedes aegypti*.** Jianwu Chen, Supaporn Likitvivatanavong, Karlygash Aimanova and Sarjeet Gill. Department of Cell Biology and Neuroscience, University of California, Riverside, CA, 92521, USA

12:00 **101 Cyt1A of *Bacillus thuringiensis* subsp. *israelensis* forms small aggregates on the midgut epithelium cell membrane of *Culex quinquefasciatus* larvae.** Maria Teresa Fernandez-Luna[1], Margaret C. Wirth[1], Elizabeth Hinde[2], Enrico Gratton[2]and Brian Federici[1]. [1]Department of Entomology, University of California, Riverside, CA 92521; [2]Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California, Irvine, CA 92679, USA

Mid-Morning Session 2 **Wednesday, 10:30 – 11:30**
Contributed Papers **Room 2**

Diseases of Beneficial Invertebrates 1

Chairs: Grant Stentiford and Elke Genersch

10:30 **102 Presence of the Israeli acute paralysis virus in honey bee collapsing colonies.** Nor Chejanovsky[1], Ron Ophir[1], Michal Sharabi[1] and Diana Cox-Foster[2]. [1]Entomology Department, The Volcani Center, Bet Dagan, POB 6, 50250 Israel; [2]Department of Entomology, The Pennsylvania State University, University Park, Pennsylvania, USA

10:45 **103 STU CBP, a new member of CBM33 family, is an important virulence factor of *Paenibacillus larvae*, the causative agent of AFB.** Eva García-González[1]; Agata Jakubowska; Salvador Herrero and Elke Genersch. [1]Länderinstitut für Bienenkunde, Molekulare Mikrobiologie und Bienenkrankenheiten, 16540 Hohen Neuendorf, Germany, [2]Department of Genetics, University of Valencia, 46100 Burjassot, Spain

11:00 **104 *Nosema ceran*ae rebounds from fumagillin control.** Wei-Fone Huang[1], Leellen F. Solter[1], Peter M. Yau[2] and Brian S. Imai[2]. [1]Illinois Natural History, Prairie Research Institute, University of Illinois, 1816 S. Oak St., Champaign, IL 61820; [2]Roy J. Carver Biotechnology Center, Protein Sciences Immunological Resource Center, 307 Noyes Laboratory, 505 S. Mathews St., Urbana, IL 61801

11:15 **105 Nitric Oxide participation in *Apis mellifera* hemocytic immune activation upon recognition of non-self and encapsulation.** Pedro Negri[1,3], Matias Daniel Maggi[1,3], Lorenzo Lamattina[2,3] and Martin Javier Eguaras[1,3]. [1]Laboratorio de Artrópodos, Universidad Nacional de Mar del Plata; [2]Instituto de Investigaciones Biológicas-CONICET, Universidad Nacional de Mar del Plata; [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Mid-Morning Session 3 **Wednesday, 10:30 – 12:00**
Contributed Papers **Auditorium 3**

Microbial Control 2

Chair: Stefan Jaronski

10:30 **106 Can *Beauveria bassiana* be a part of strawberry IPM in California Central Coast?** Surendra K. Dara. University of California Cooperative Extension, San Luis Obispo, CA 93401, USA

10:45 **107 Compatibility of fruit fly attractants with *Metarhizium anisopliae* for the management of *Bactrocera invadens*, an invasive pest of horticulture in Africa.** Sunday Ekesi, Samira Mohamed, M. Fathiya Khamis and K. Nguya Maniania. International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772 - 00100, Nairobi, Kenya

11:00 **108 Use of *Metarhizium anisopliae* to control the leafhopper: characterization of two major commercial production areas of sugar cane in Brazil.** Adriana Regina Generoso, Tatiana Bernardino Ferratto, Mariana Vieira Christal, Michele Cristina Lanza and Mariana Taglietto de Oliveira. FATEC - Faculty of Technology of São José do Rio Preto, Brazil

11:15 **109 Research on *Metarhizium* for wireworm management – retrospective and foresight.** Todd Kabaluk. Agriculture and Agri-Food Canada, Agassiz, British Columbia

11:30 **110 Influence of plant culture conditions on efficacy of foliar applications of entomopathogenic fungi against western flower thrips.** Stephen P. Wraight and Mark E. Ramos. USDA-ARS Robert W. Holley Center for Agriculture and Health; Biological Integrated Pest Management Research Unit, Ithaca, NY 14853 USA

11:45 **111 Development of *Metarhizium anisopliae* strain F52 in North America and Europe.** Jarrod Leland Novozymes Biologicals, Inc., 5400 Corporate Circle, Salem VA 24153 United State

Mid-Morning Session 4 **Wednesday, 10:30 – 11:45**
Contributed Papers **Room 1**

Nematodes 2

Chairs: Patricia Stock and Selcuk Hazir

10:30 **112 STU *Delia platura,* Meigen 1826 (Diptera: Anthomyiidae) control with entomopathogenic nematode Steinernema sp3 JCL027 in Cota (Cundinamarca), Colombia.** Carolina Jaramillo[1] and Adriana Sáenz Aponte[2]. [1]Pontificia Universidad Javeriana. Bogotá, Colombia; [2]Unit of Ecology and Systematics – UNESIS, Biological Control Laboratory, Pontificia Universidad Javeriana, Cra 7 N° 43-82, place 54, Of 200. Bogotá, Colombia

10:45 **113 Insect host diet and its impact on the fitness of entomopathogenic nematodes and their symbiotic bacteria.** S. Patricia Stock and Vitoria Miranda. Department of Entomology, The University of Arizona, Tucson, AZ, USA

11:00 **114 Contributions of cognate and non-cognate symbionts to nematode host fitness.** S. Patricia Stock[1], Ming-Min Lee[1]and E. Dehaven[2]. [1]Department of Entomology, The University of Arizona, Tucson, AZ, USA; [2]Department of Molecular and Cellular Biology, The Universiity of Arizona, Tucson, AZ, USA

11:15 **115 Olfactory response of the mite, *Sancassania polyphyllae*, to cadavers and tissues with and without entomopathogenic nematodes: impact on biological control.** Selcuk Hazir[1], Ibrahim Cakmak[2], Derya Asici[1], Mehmet Karagoz[2] and Harry K. Kaya[3]. [1]Adnan Menderes University, Faculty of Arts and Science, Department of Biology, 09010 Aydin, Turkey, [2] Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, 09010 Aydin, Turkey, [3]Department of Nematology, University of California, One Shields Avenue, Davis, CA 95616, USA

11:30 **116 Evolutionary relationships between *Deladenus* nematodes parasitizing northeastern North American *Sirex* species.** Elizabeth Erin Morris[1], Ryan M. Kepler[1], Stefan J. Long[1], David W. Williams[2], and Ann E. Hajek[1] . [1]Department of Entomology, Cornell University, Ithaca, NY 14853-2601; [2]USDA-APHIS PPQ, Otis Lab, Buzzards Bay, MA 02542

Mid-Morning Session 4 **Wednesday, 10:30-11:45**
Contributed Papers **Auditorium 2**

Viruses 3

Functional Genomics I
Chairs: Martin Erlandson and Zhihong Hu

10:30 **117 STU Protein tyrosine phosphatase-induced hyperactivity is an evolutionarily conserved strategy of baculoviruses to manipulate lepidopteran host behavior.** Stineke van Houte, Vera I.D. Ros, Just M. Vlak and Monique M. van Oers. Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, the Netherlands

10:45 **118 STU SeMNPV genotypic variants with increased replication efficiency in cultured *Spodoptera exigua* cells lack a gene with pro-apoptotic activity.** Amaya Serrano[1,2], Stineke van Houte[2], Primitivo Caballero[1], Just M. Vlak[2], Monique M. van Oers[2] and Gorben Pijlman[2]. [1] Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, 31192 Mutilva Baja, Navarra, Spain. [2] Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

11:00 **119 STU Determination of the role *me53/*ME53 plays in both early and late phases in the baculovirus replication cycle.** Yang Liu and Peter Krell. Department of Molecular and Cellular Biology, University of Guelph, ON, Canada N1G 2W1

11:15 **120 The distribution and phosphorylation of the basic protein P6.9 of *Autographa californica* nucleopolyhedrovirus.** XiaoXiao Liu, Zhixin Fang, Meijin Yuan, Kai Yang and Yi Pang. State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

11:30 **121 STU Acid activation of the budded virus fusion protein F of *Spodoptera exigua* multicapsid nucleopolyhedrovirus.** Qiushi Wang[1,2], Michael van de Weijer[1], Tom van den Hoeven[1], Monique M. van Oers[2], Just M. Vlak[2], Peter Rottier[1] and Berend-Jan Bosch[1]. [1] Virology Division, Faculty of Veterinary Medicine, Department of Infectious Disease and Immunology, Utrecht University; [2] Laboratory of Virology, Plant Sciences Group, Wageningen University

11:45 **122 Structure-based functional models of fusion peptide of baculovirus envelope fusion protein F.** Manli Wang[2], Danyun Zeng[1], Ying Tan[2], Jingwen Xiong[1], Fei Deng[2], Maili Liu[1], Zhihong Hu[2], Ling Jiang[1] and Hualin Wang[2] . [1]Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, China; [2] State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

12:00 **123 Incorporation of GP64 into *Helicoverpa armigera* NPV enhances virus infectivity both *in vivo* and *in vitro.*** Shu Shen , Yinyin Gan , Manli Wang, Zhihong Hu, Hualin Wang, and Fei Deng. State Key Laboratory of Virology Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P. R. China

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12:45 **5 K Race**

12:30 – 14:15 **LUNCH** **Setting up Poster Session 2 (Room 3)**

Journal of Invertebrate Pathology Editorial Board Meeting **Wednesday, 12:30 – 14:30 Room 2**

Afternoon Session 1 **Wednesday, 14:45 – 16:30**
Symposium VIII - Bacteria Division **Auditorium 2**

Bacterial topics of interest to Latin America

Organizers: Wiliam Moar and Alicia Sciocco-Cap

14:45 **124 Assessment of the high-dose concept and level of control provided by MON 87701 × MON 89788 soybean in Brazil.** Samuel Martinelli[1], Luciano B Fonseca[1], Geraldo U Berger[1] and Graham P Head[2]. [1]Monsanto of Brazil Ltda, São Paulo, Brazil, [2] Monsanto LLC, St Louis,Missouri, USA

15:10 **125 Vip3A, a novel mode of insecticide action to improve productivity and sustainability.** Alejandro Tozzini. Syngenta, Argentina

15:35 **126 Systemic utilization of *Bacillus thuringiensis* – a new tool for pest control.** Rose Monnerat. Embrapa Recursos Genéticos e Biotecnología, Brazil

15:55 **127 *Bacillus thuringiensis* crystal proteins as cures for intestinal roundworms.** Yan Hu, Brian Ellis, Jillian Sesar, Melanie Miller, Ying Yiu and Raffi V. Aroian. Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA 92093-0322, USA

Afternoon Session 2 **Wednesday, 14:45 – 16:45**
Symposium IX – DBI and Microsporidia Divisions **Room 2**

New insights into host-pathogen interaction in the Microsporidia

Organizers: Dörte Goertz and Grant Stentiford

14:45 **128 Investigating the secretome of diverse microsporidia.** Bryony Williams[1], Grant Stentiford[2] and Scott Campbell[1]. [1]Biosciences, University of Exeter, Devon, UK.; [2]CEFAS, Weymouth, Dorset, UK

15:45 **129 Genomic insights into the interactions of the microsporidian parasites *Nosema* and their honey bee hosts.** Yan Ping (Judy) Chen[1], S. Jeffery Pettis[1], Yan Zhao [2], Scott R. Cornman [1] and D. Jay Evans [1]. [1]Bee Research Laboratory, US Department of Agriculture-Agricultural Research Service, Beltsville, MD, USA; [2]Molecular Plant Pathology Laboratory, US Department of Agriculture-Agricultural Research Service, Beltsville, MD, USA

Afternoon Session 3 **Wednesday, 14:45 – 16:30**
Contributed Papers **Auditorium 4**

Viruses 4

Insect viruses

Chairs: Zihni Demirbag and Rollie Clem

14:45 **130 STU Structure, protein composition, morphogenesis and cytopathology of *Glossina pallidipes* Hytrosavirus.** Henry M. Kariithi[1, 2, 3], Jan van Lent[1], Monique M. van Oers[1], Adly M.M. Abd-Alla[2], İkbal Agah İnce [1] and Just M. Vlak[1]. [1]Laboratory of Virology, Wageningen University, The Netherlands, [2]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria, [3]Biotechnology Centre, Kenya Agricultural Research Institute, Kaptagat Rd, Loresho, Nairobi, Kenya

15:00 **131 Impact of salivary gland hypertrophy virus infection on the mating success of male *Glossina pallidipes*: consequences for the sterile insect technique.** Gratian N. Mutika, Carmen Marin, Andrew G. Parker, Marc J.B. Vreysen and Adly M. M. Abd-Alla. Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria

15:15 **132 Molecular and Functional Analysis of ORF AMV133 Encoded by *Amsacta moorei* Entomopoxvirus (AmEPV).** Emine Demir, Kazim Sezen, Zihni Demirbag. Karadeniz Technical University, Faculty of Sciences, Department of Biology, 61080, Turkey

15:30 **133 The effect of expressing apoptosis-regulating genes on alphavirus replication in the mosquito vector *Aedes aegypti.*** Katelyn O'Neill and Rollie J. Clem. Division of Biology, Kansas State University, Manhattan, KS USA

15:45 **134 Replication biology of Providence virus (Family: *Carmotetraviridae*): a plant virus with an animal virus capsid that replicates in insects?** James R Short, Ritah Nakayinga, Mpho Peter and Rosemary A. Dorrington. Department of Biochemistry, Microbiology and Biotechnology. Rhodes University, PO Box 94, Grahamstown, 6140, South Africa

16:00 **135 Suppression of RNA silencing by Wuhan Nodavirus.** Nan Qi, Congyi Zheng, Jiamin Zhang, Xi Zhou and Yuanyang Hu. State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei 430072 China

16:15 **136 The Drosophila RNAi machinery not only provides antiviral defense against RNA viruses but also DNA viruses.** Alfred W. Bronkhorst[1], Koen W.R. van Cleef[1], Nicolas Vodovar[2], I. Agah Ince[3], Hervé Blanc[2], Just M. Vlak[3], Maria-Carla Saleh[2] and Ronald P. van Rij[1]. [1]Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen Centre for Molecular Life Sciences, Nijmegen Institute for Infection, inflammation and Immunity, 6500 HB Nijmegen, The Netherlands; [2]Institut Pasteur,. Viruses and RNA interference Group and Centre National de la Recherche Scientifique, URA 3015, 75015 Paris, France; [3]Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

Afternoon Session 4 **Wednesday, 14:45 – 16:00**
Contributed Papers **Auditorium 3**

Fungi 3

Chairs: Helen Hesketh and Ingeborg Klingen

14:45 **137 Elevated spring temperatures will impact fungal disease in gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), larvae.** Joanna J. Fisher, Keith M. Ciccaglione and Ann E. Hajek. Department of Entomology, Cornell University, Ithaca NY 14853-2601, USA

15:00 **138 Importance of spore discharge (numbers, distance and direction) of *Neozygites floridana* for epidemic development in *Tetranychus* populations.** Ingeborg Klingen[1], Silje Stenstad Nilsen[1,2] Rennan Almeida Da Silva[3], Vitalis W. Wekesa[1, 3, 4] and Italo Delalibera Jr[3]. [1]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division. [2]Norwegian University of Life Sciences, Department of Plant and Environmental Sciences. [3]ESALQ, University of São Paulo, Department of Entomology and Acarology. [4]Kenya Polytechnic University College (A constituent college of the University of Nairobi), Department of Biological Science and Technology

15:15 **139 Degeneration of wild-type and transgenic strains of *Beauveria bassiana.*** Zengzhi Li, Xiaoqing Tang, Jinzhu Xu, and Liming Wang. Center for Entomogenous Fungi, Anhui Agricultural University, Hefei, Anhui 230036, China

15:30 **140 Defense reactions of *Leptinotarsa decemlineata* larvae under combined treatments by fungus *Metarhizium anisopliae* s.l., bacteria *Bacillus thuringiensis tenebrionis* and organophosphorus insecticide.** Olga. N. Yaroslavtseva, Ivan M. Dubovskiy, Vadim Yu Kryukov, Elena V. Surina, Galina V. Benkovskaya and Viktor V. Glupov. Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

15:45 **141 Dietary effects on enzymatic immunity of migrating Mormon crickets to fungi and bacteria.** Robert B. Srygley. USDA-Agricultural Research Service, Sidney Montana USA

16:30 – 16:45 **BREAK**

Poster Session 2 **Wednesday, 16:45 – 18:45**
Room 3

Bacteria

B-18 **Characterization and colonization inside the plants *in vitro* of endophytic *B. thuringiensis* from sugar cane.** Marise Tanaka Suzuki[1], Carmen Sara Hernández-Rodríguez[2], Juan Ferré[2] and Araújo Welington Luiz[3]. [1]Departament of Biology Applied of Agriculture - Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP/FCAV, Jaboticabal/Brazil; [2]Departament of Genetics - Universitat de València/Burjassot, Valencia/Spain; [3]Departament of Microbiology - Universidade de São Paulo, São Paulo/Brazil

B-19 **The effect of gamma sterilization on the insecticidal toxicity of engineered and conventional *Bacillus thuringiensis* strains.** Shifeng Sun [1] Jing Fan,[1] Zhongshan Cheng,[2] and Yi Pang[1*]. [1]State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, People's Republic of China; [2]Department of Microbiology, the University of Hong Kong, Hong Kong, People's Republic of China

B-20 **The importance of antibiosis for the successful reproduction of *Bacillus thuringiensis* in insects.** Ben Raymond. Royal Holloway University of London, Egham, Surrey, TW20 0EX, UK

B-21 **Effects of gut bacteria to the insecticidal activity of *Bacillus thuringiensis* on *Helicoverpa armigera.*** Li Mingshun, Zhang Hao, Xue Yan, Hou Yanfei and Yu Ziniu State Key Laboratory of Agricultural icrobiology, Huazhong Agricultural University, Hubei, Wuhan 430070, P. R. China

B-22 **Immune response of *Galleria mellonella* (Lepidoptera, Pyralidae) larvae during bacterial infection by *Bacillus thuringiensis.*** Ekaterina Grizanova*, Ivan Dubovskiy and Viktor Glupov. Institute of Systematics and Ecology of Animals, Siberian Branch Russian Academy of Sciences, Novosibirsk, Russia

B-23 **Characterization of *vip* genes and toxicity of *Bacillus thuringiensis* against *Spodoptera frugiperda.*** Camila da Silva Fernandes[2], Thais Barros Rodrigues[1], Rosane Bezerra da Silva[1], Arthur Augusto Gonçalves Torres[2], André Henrique Campelo Mourão[2], Kátia Gisele Brasil Boregas[3] and Fernando Hercos Valicente[3]. [1]Federal University of Lavras; [2]Federal University of São João Del Rei; [3]Embrapa Maize and Sorghum Research, Brazil

B-24 **Identification of Coleoptera and Lepidoptera-specific *vip* genes in Argentinean and exotic *Bacillus thuringiensis* strains.** Diego Sauka, María Inés Onco, Sonia Rodríguez, Melisa Pérez and Graciela Benintende. Insumos Bacterianos. Instituto de Microbiología y Zoología Agrícola (IMYZA), Instituto Nacional de Tecnología Agropecuaria (INTA). Buenos Aires, Argentina

B-25 **Asparagine substitution in block 3 of *Bacillus thuringiensis* crystal protein Cry5Ba improved the crystal solubility and increased the toxicity against *Caenorhabditis elegans.*** Fenshan Wang, Yingying Liu, Fengjuan Zhang, Lujun Chai, Lifang Ruan, Donghai Peng and Ming Sun. State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China

B-26 **Characterization of an active partition system for the *Bacillus sphaericus* mosquitocidal plasmid pBsph.** Yong Ge, Xiaomin Hu, Yiming Wu and Zhiming Yuan. Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

B-27 **Generation of mariner-based transposon insertion mutant library of *Bacillus sphaericus* 2297 and investigation of genes involved in sporulation and mosquito-larvicidal crystal protein synthesis.** Yiming Wu, Xiaomin Hu, Yong Ge, Dasheng Zheng and Zhiming Yuan. Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

B-28 **Characterization of a *Bacillus thuringiensis* strain native from Argentina toxic against mosquito species.** Corina M. Berón, María E. Vidal-Domínguez and Leonardo M. Díaz-Nieto. Centro de Estudios de Biodiversidad y Biotecnología – Centro de Investigaciones Biológicas – Fundación para Investigaciones Biológicas Aplicadas, Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Vieytes 3103, 7600 Mar del Plata, Argentina

B-29 **Enterotoxigenic and psychrotrophic but not entomopathogenic properties of environmental *Bacillus thuringiensis* isolates correlate with the phylogenic relatedness.** Izabela Swiecicka, Elwira Maciuszko. Department of Microbiology, University of Bialystok, 20B Swierkowa Street, PL15-950 Bialystok, Poland

B-30 **Experimental evidence supporting the pore-forming model of the mechanism of action of 3d-Cry toxins.** Isabel Gómez, Carlos Muñoz-Garay, Liliana Pardo, Helena Porta, Claudia Rodriguez, Jorge Sanchez, Luis E.Zavala, Violeta Matus, Leivi Portugal, Josue Ocelotl, Fernando Zuñiga, Daniela Carmona, Mario Soberón and Alejandra Bravo. Instituto de Biotecnología, Universidad Nacional Autónoma de México, Mexico

B-31 ***Bacillus thuringiensis* and plants: an *in vitro* model to study interactions.** J. Cristian Vidal-Quist, Hilary Rogers, Eshwar Mahenthiralingam and Colin Berry. School of Biosciences, Cardiff University, UK

B-32 ***Bacillus thuringiensis* strains for control of pest in Brazil**. Gislayne T. Vilas Boas[1], Luisa C. F. Helene, Pedro M. O. J. Neves[1], Kelly C. K. Silva[1], Fabiane Cunha[2], Flavio Moscardi[1;2], Daniel R. Sosa-Gomez[3], Rose Monnerat[4], Talita M. Alexandre[5] and Luis Francisco A. Alves[5]. [1]State University of Londrina, 86051-970 - Londrina, PR, Brazil; [2] UNOESTE, Presidente Prudente, SP, Brazil; [3]EMBRAPA-Soja – Londrina, Pr, Brazil; [4]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil; [5]UNIOESTE, Cascavel, PR, Brazil

B-33 **Shell disease by *Vibrio* sp. in grapsid crabs from Bahía Blanca estuary, Argentina.** Sergio Martorelli, Pilar Alda, Paula Marcotegui, Martin Montes and Javier Panei. Centro de Estudios Parasitológicos y Vectores (CEPAVE), CONICET-CCT La Plata, Calle 2 No. 584, La Plata 1900, Buenos Aires, Argentina

B-34 **STU Cloning and expression of a novel *cry1I* gene from *Bacillus thuringiensis* isolates and its toxicity against *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae) and *Helicoverpa armigera* Hübner (Noctuidae: Lepidoptera).** H.M. Mahadeva Swamy[1], R. Asokan[1], Geetha G. Thimmegowda3, D.K. Arora[2], S.N. Nagesha[1] and Riaz Mahmood[4]. [1]Division of Biotechnology, Indian Institute of Horticultural Research (IIHR), Hessarghatta lake post, Bangalore 560089, Karnataka. [2]National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau Nath Bhajan, 275101, Uttar Pradesh. [3]Division of Entomology & Nematology, Indian Institute of Horticultural Research (IIHR), Hessarghatta lake post, Bangalore 560089, Karnataka. [4]Post-Graduate Department of Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Jnanasahayadri, Shankaraghatta, Shimoga 577451 Karnataka, India

Poster Session 2
Fungi

Wednesday, 16:45 – 18:45
Room 3

F-20 ***Sclerotinia sclerotiorum* white mold inhibition by volatile metabolites of entomopathogenic fungi.** Ciro H. Sumida, Idenize P. Orsini[1], Kelly C. C. Silva[1], Beatriz Kraemer[1] and Pedro M. O. J. Neves[1] [1]Agronomy Department, Microbial Insects Control Laboratory, State University of Londrina, 86051-970 - Londrina, Paraná, Brazil

F-21 **Influence of successive *in vitro* cultivation of *Beauveria bassiana* (Bals.) Vuill on virulence to *Alphitobius diaperinus.*** Patricia H. Santoro[1], Pedro M. O. J. Neves[1], Janaina Zorzetti[1] and Kelly C. K. Silva[1] [1]Agronomy Department, Microbial Insects Control Laboratory, State University of Londrina, 86051-970 - Londrina, Paraná, Brazil

F-22 **Entomophthorales fungi (Zygomycetes) pathogens of aphids (Hemiptera: Aphididae) associated with cereal crops in Argentina.** Romina G Manfrino[1,2]; Claudia C. López Lastra [2] and César E. Salto[1]. [1] Instituto Nacional de Tecnología Agropecuaria (INTA). Área Investigación Agronomia. Protección Vegetal. Ruta Nacional 34, Km. 227. Rafaela (2300), Santa Fe, Argentina; [2] Centro de Estudios Parasitológicos y de Vectores (CEPAVE). UNLP-CONICET. Calle 2, nro 584. La Plata (1900). Buenos Aires, Argentina

F-23 **Morphological characterization of *Hirsutella citriformis* species infecting *Diaphorina citri* Kuwayama in Mexico.** Orquídea Pérez-González[1], María Guadalupe Maldonado-Blanco[1], Raúl Rodríguez-Guerra[2], José Isabel López-Arroyo[2] and Myriam Elías-Santos[1]. [1]Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. Av. Pedro de Alba y Manuel L. Barragán s/n Ciudad Universitaria, C. P. 66450, A. P. 414 y 2790. San Nicolás de los Garza, Nuevo León, México. [2]Instituto de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental General Terán, Carr. Montemorelos-China, Km 31, C.P. 67400, Gral. Terán, Nuevo León, Mexico

F-24 ***Metarhizium anisopliae* and *Beauveria bassiana* blastospores obtained in submerged culture against *Aedes aegypti* larvae and adults.** María Guadalupe Maldonado-Blanco, Johanna Lizzette Gallegos-Sandoval, Gabriela Fernández-Peña, Carlos Francisco Sandoval-Coronado and Myriam Elías-Santos. Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. Av. Pedro de Alba y Manuel L. Barragán s/n Ciudad Universitaria, C. P. 66450, A. P. 414 y 2790. San Nicolás de los Garza, Nuevo León, México. (mgpemald@hotmail.com)

F-25 **Relative production of Metarhizium propagules and their potential as human pathogens.** Todd Kabaluk[1], Benoit Szegedi[2], Jeanne Boulard[3], Nina Lachia[4] and Mauricio Rivera[5]. [1]Agriculture and Agri-Food Canada, Agassiz, BC, [2]Universitaire de Technologie Claude Bernard – Lyon 1, Lyon France, [3]Institut Universitaire de Technologie, Lyon, France, [4]Montpellier SupAgro, Monpellier, France, [5]Fundacioón Hondureña de Investigación Agrícola; Honduras

F-26 **Distribution of Metarhizium Species in Relation to Ecoregions of the North American Subcontinent.** Todd Kabaluk[1], Doug Inglis[2], Grant Duke[2], Mark Goettel[2], Cam Kenny[3] and Lerry Lacey[4]. Agriculture and Agri-Food Canada; [1]Agassiz, British Columbia; [2]Lethbridge, Alberta; [3]Saskatoon, Saskatchewan; [4]United States Department of Agriculture, Yakima, Washington; USA

F-27 **Response of *Beauveria bassiana* and *Metarhizium* spp. vegetative cultures to transient high temperatures.** Stefan T. Jaronski, USDA ARS Northern Plains Agricultural Research Laboratory, Sidney MY USA 59270

F-28 **HURRICANE WARNING! How changed nomenclatural rules affect fungal entomopathogens.** Richard A. Humber. USDA-ARS Biological Integrated Pest Management, RW Holley Center for Agriculture & Health, 538 Tower Road, Ithaca, NY 14853, USA

F-29 **Phylogenetic reclassification raises new respect–and a new phylum!–for Entomophthorales.** Richard A. Humber[1], Andrii Gryganskyi[2] and Rytas Vilgalys[2]. [1] USDA-ARS Biological Integrated Pest Management, RW Holley Center for Agriculture & Health, 538 Tower Road, Ithaca, NY 14853, USA; [2] Dept. of Biology, Duke University, Durham, NC 27708, USA

F-30 **Pathogenicity of *Metarhizium anisopliae* (Metchn.) Sorok on *Blattella germanica* (Linnaeus) (Blattodea: Blattellidae) and *Periplaneta fuliginosa* (Seville) (Blattodea: Blattidae), in Argentina.** Alejandra C. Gutierrez[1,2], Pablo M. López[1], Juan J. García[1,2] and Claudia C.López Lastra[1,3]. [1]Centro de Estudios Parasitológicos y de Vectores (CEPAVE) [2](CIC-UNLP) [3](CONICET-UNLP). Calle 2 Nº 584, CP 1900, La Plata, Buenos Aires, Argentina

F-31 **Lipolytic and proteolytic activities of *Metarhizium anisopliae sensu lato* isolates associated to its virulence on *Rhipicephalus microplus* ticks.** Wendell Marcelo de Souza Perinotto[1], Patrícia Silva Golo[1], Lucélia Santi[2], Marilene Henning Vainstein[2], Walter OrlandoBeys da Silva[2], Cristiane Martins Cardoso Salles[3] and Vânia Rita Elias Pinheiro Bittencourt[1]. [1]Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil; [2]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; [3]Departamento de Química, Instituto de Ciências Exatas, UFRRJ, Seropédica, RJ, Brazil

F-32 **Conidial Pr1 activity of *Metarhizium anisopliae*: a comparative study of the proteolytic activity of conidia produced on artificial medium or tick cadavers.** Patrícia Silva Golo[1], Wendell Marcelo de Souza Perinotto[1], Mariana Guedes Camargo[1], Isabele da Costa Angelo[1], Simone Quinelato, Éverton Kort KampFernandes[2] and Vânia Rita Elias Pinheiro Bittencourt[1]. [1]Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro- UFRRJ; [2]Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás-UFG

F-33 **Susceptibility of *Galleria mellonella* larvae parasitized by ectoparasitoid *Habrobracon hebetor* to anamorphic entomopathogenic ascomicetes** Vadim Yu. Kryukov, Natalia A. Kryukova and Viktor V. Glupov Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

F-34 **Anti-fungal activity of protein extracts on the *Bipolaris oryzae* and *Gerlachia oryzae* phytopathogens.** Neiva Knaak[1,2], Letícia Dias da Silva[1], Tiago Finger Andreis[1] and Lidia Mariana Fiuza[1,2]. [1]UNISINOS, Laboratory of Microbiology and Toxicology. CEP 93001-970, São Leopoldo, RS/Brazil; [2]IRGA/EEA, Rice Experiment Station, CEP 94930-030, Cachoerinha, RS/Brazil

F-35 **Diversity of *Metarhizium* spp. isolates from Western and Central United States.** Éverton K. K. Fernandes[1,2], Chad A. Keyser[1], Jer Pin Chong[3], Drauzio E. N Rangel[4], Nelson Foster[5], Larry Jech[5], Stephen Rehner[6], Karen Mock[3] and Donald W.Roberts[1]. [1]Department of Biology, Utah State University, Logan, UT, USA; [2]Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil,; [3]Department of Wildland Resources, Utah State University, Logan, UT, USA; [4]Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, SP, Brazil; [5]USDA/APHIS/PPQ/CPHST Lab, Phoenix, AZ, USA; [6]Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland, USA

F-36 **Pathogenicity and horizontal transmission of entomopathogenic fungi to *Diaphorina citri* (Hemiptera: Psyllidae).** Celeste P. D'Alessandro, Marcos R. Conceschi, Jessica Pampolini, Bruna Campos and Italo Delalibera Jr. Department of Entomology and Acarology, ESALQ, University of São Paulo, Av. Pádua Dias 11, C.P. 9, Piracicaba, São Paulo, Brazil

F-37 **Fungi associated with epizootic of hemlock woolly adelgid, *Adelgid tsugae* Annand (Hemiptera: Adelgidae).** Gouli Vladimir, Gouli Svetlana, Skinner Margaret and Parker Bruce. Entomology Research Laboratory, University of Vermont, Burlington, Vermont, 05405-0105, USA

Poster Session 2

Wednesday, 16:45 – 18:45

Room 3

Microbial Control

MC-24 **Microbial control of *Pseudoplusia includens* (Walker) and *Anticarsia gemmatalis* Hübner with their viruses, PsiSNPV and AgMNPV.** Daniel Ricardo Sosa-Gomez. Embrapa Soybean, Cx. P. 231, Londrina, PR, Brazil

MC-25 **Evaluation of *Cydia pomonella* granulovirus (CpGV) in combination with Rynaxypyr and Metoxyphenozide for codling moth control in walnuts orchards in Catamarca, Argentina.** Graciela M. Quintana[1], Juan J. Cólica[2], Omar M. Farinon[1] and Rubén F. La Rossa[1]. [1]IMYZA-CCVyA-INTA Castelar. CC25 (1712) Castelar, Argentina; [2]AER INTA Andalgalá. Catamarca, Argentina

MC-26 **Effect of *Bacillus thuringiensis* on the phytophagous activity of *Podisus nigrispinus* on kale leaves and on its consumption of *Plutella xylostella* larvae.** Alessandra Marieli Vacari, Gustavo Oliveira de Magalhães, Valeria Lucas de Laurentis, Haroldo Xavier Linhares Volpe, Ana Carolina Pires Veiga, Sergio Antonio De Bortoli and Ricardo Antonio Polanczyk. Laboratory of Biology and Insect Rearing (LBIR), Department of Crop Protection, Unesp, Jaboticabal, Sao Paulo, Brazil

MC-27 **Efficacy of an aqueous suspension of *Bacillus thuringiensis* var. *israelensis* against *Aedes vexans* larvae in Xinjiang Irtysh river lower reach área.** Dong Tian[1], Quanxing Cai[1], Jingchang Zhang[2], Yuehua Jing[2], Zhiming Yuan[1]and Jianping Yan[1]. [1]Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

MC-28 **Leaf consumption of *Plutella xylostella* assayed with *Bacillus thuringiensis.*** Sergio Antonio De Bortoli, Valeria Lucas de Laurentis, Haroldo Xavier Linhares Volpe, Ana Carolina Pires Veiga, Alessandra Marieli Vacariand Ricardo Antonio Polanczyk. Laboratory of Biology and Insect Rearing (LBIR), Department of Crop Protection, Unesp, Jaboticabal, Sao Paulo, Brazil

MC-29 **Encapsulation of *Bacillus thuringiesis* Vip3A toxin in *Pseudomonas fluorescens* as a way to develop new spray bioinsecticides.** Carmen Sara Hernández-Rodríguez[1], Iñigo Ruiz de Escudero[2,3], Primitivo Caballero[2,3]and Juan Ferré[1]. [1]Departamento de Genética, Facultad de CC. Biológicas, Universidad de Valencia, Valencia, Spain; [2]Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadía, 31192 Mutilva Baja, Navarra, Spain; [3]Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, 31006 Pamplona, Spain

MC-30 **Hemicellulose compatibility to *Beauveria bassiana* and *Metarhizium anisopliae* fungi and their effect on development parameters of the entomopathogens.** Inajá. M Wenzel[1,2], Antonio Batista Filho[2], Moacir R. Forim[1] and Eveline S. Costa[1]. [1]Federal University of São Carlos/Chemistry Departament/Natural Products Laboratory/São Carlos city, São Paulo state, Brazil. [2]Biological Institute/Bilogical Control Laboratory/ Campinas city, São Paulo state, Brazil

MC-31 **Determination of Lethal Concentration 50 of *Beauveria bassiana* and *Metarhizium anisopliae* fungi to the sugarcane borer *Diatraea saccharalis*.** Inajá. M. Wenzel[1,2], Antonio Batista Filho[2], Moacir, R.Forim[1]. [1]Federal University of São Carlos/Chemistry Departament/Natural Products Laboratory/São Carlos City, São Paulo State, Brazil [2]Biological Institute/Bilogical Control Laboratory/ Campinas City, São Paulo State, Brazil

MC-32 **Effect of formulation on the oily conidial viability of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes).** Aline Menezes dos Santos[1], Marcelo da Costa Mendoça[2] and Ana Amélia Moreira Lira[3]. [1]Programa de Pós-Graduação em Biotecnologia, Universidade Federal de Sergipe, Cidade Universitária Prof. José Aloísio de Campos, CEP 49100-000, São Cristóvão, SE; [2]Empresa de Desenvolvimento Agropecuário de Sergipe/Embrapa Tabuleiros Costeiros, Av. Carlos Rodrigues da Cruz, s/n, Aracaju,SE, CEP: 49.080-190; [3]Departamento de Farmácia, Universidade Federal de Sergipe, Cidade Universitária Prof. José Aloísio de Campos, CEP 49100-000, São Cristóvão, SE Brazil

MC-33 **Formulation of entomopathogenic fungus, *Beauveria bassiana* (Vuill.) in alginate matrix.** Ísis Tatiana Borges Jordão Braga[1], Marcelo da Costa Mendoça[2] and Ana Amélia Moreira Lira[3]. [1]Programa de Pós-Graduação em Biotecnologia, Universidade Federal de Sergipe, Cidade Universitária Prof. José Aloísio de Campos, CEP 49100-000, São Cristóvão, SE; [2]Empresa de Desenvolvimento Agropecuário de Sergipe/Embrapa Tabuleiros Costeiros, Av. Carlos Rodrigues da Cruz, s/n, Aracaju,SE, CEP: 49.080-190; [3]Departamento de Farmácia, Universidade Federal de Sergipe, Cidade Universitária Prof. José Aloísio de Campos, CEP 49100-000, São Cristóvão, SE Brazil

MC-34 **Management of *Meloidogyne enterolobii* in culture of guava, in Brazilian semiarid region, with the fungi *Paecilomyces lilacinus* and *Trichoderma* spp.** Alexandre M. Guimarães, Rita C.M. Santin, Marcia E. Silva, Andressa M.S. Souza, Isabel C.P. Paz and Ainda T. S. Matsumura[1]. [1]ICB BIOAGRITEC Ltda, Rua Arabutã, 386, Bairro Navegantes, Porto Alegre/RS, CEP 90.240-470. Brazil

MC-35 **Molluscicidal activity of *Bacillus thuringiensis* against golden mussel, *Limnoperna fortune.*** Isabel C.P Paz[1], Daniel Pereira[1], Andressa M.S.Souza[1]; Marise T.Suzuki[2], João Lúcio Azevedo[2], Paulo S.Formagio[3], Maria Cristina D. Mansur[1] and Maria Teresa R.Rodriguez[1]. [1] Fundação Luiz Englert/ Centro de Ecologia, UFRGS. Av. Bento Gonçalves, 9500, setor 4, bloco 43411, sala 118, Bairro Agronomia, Porto Alegre/RS, CEP 91570-000; [2] Laboratório de Genética de Microrganismos, ESALQ/USP; [3] Furnas- Centrais Elétricas S/A, Departamento de Produção de Minas/ EHPF. Brazil

MC-36 **Identification of serpins in hemolymph of *Rhipicephalus microplus* infected by entomopathogenic fungi.** Isabele da Costa Angelo[1], Patrícia Silva Golo[1], Wendell Marcello de Souza Perinotto[1], Camargo Mariana Guedes[1], Simone Quinelato[1], Fillipe Araújo de Sá[1], Márcia Soares[2] and Vânia Rita Elias Pinheiro Bittencourt[1]. [1]Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil. [2]Departamento de Química, Centro de Tecnologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

MC-37 **Comparative study between formulations of entomopathogenic nematode-infected cadavers to control *Rhipicephalus microplus* ticks.** Caio Márcio de Oliveira Monteiro[1], Patrícia Silva Golo[1], Renata da Silva Matos[2], Laryssa Araújo[2], Wendell Marcelo de Souza Perinotto[1], Márcia Cristina de Azevedo Prata[3], Vânia Rita Elias Pinheiro Bittencourt[1], Claudia Dolinski[4] and John Furlong[3]. [1]Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brasil; [2]Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil; [3]Embrapa Gado de Leite, Juiz de Fora, MG, Brazil; [4]Universidade Estadual Norte Fluminense, Campos dos Goytacazes, RJ, Brazil

MC-38 **Advances in the research about mycoacaricides against RMSF vectors in Latin America.** Walmirton B. D'Alessandro[1], Macsuel C. Barreto[1], Juscelino Rodrigues[1], Fabrício M. Alves[1], Tássio L. Tavares[1], Richard A Humber[2], Éverton KK Fernandes[1]and Christian Luz[1]. [1]DMIPP, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia, GO, Brazil; [2]USDA-ARS Biological Integrated Pest Management Research, Robert W. Holley Center for Agriculture and Health, Ithaca, NY, USA

Poster Session 2 Microsporidia

Wednesday, 16:45 – 18:45
Room 3

M-01 **Spread of *Nosema lymantriae* in experimental gypsy moth populations – first results.** Dörte Goertz[1] and Milan Zubrik[2]. [1.]University of Natural Resources and Life Sciences, Department of Forest- and Soil Sciences, Institute of Forest Entomology, Forest Pathology and Forest Protection, Hasenauer Str. 38, 1190 Vienna, Austria; [2.]National Forest Centre, T. G. Masaryka st. 22, SK-96092 Zvolen, Slovak Republic

M-02 **Effects of *Bacillus thuringiensis* on a co-occurring microsporidian infection in *Lymantria dispar.*** Dörte Goertz[1], Martina Mayrhofer[1] and Gernot Hoch[1,2]. [1]University of Natural Resources and Life Sciences, Department of Forest- and Soil Sciences, Institute of Forest Entomology, Forest Pathology and Forest Protection, Hasenauer Str. 38, 1190 Vienna, Austria; [2]Institute of Forest Protection, BFW – Federal Research Centre for Forests, Seckendorff-Gudent-Weg 8, 1131 Vienna, Austria

M-03 **"Cotton shrimp" disease in the freshwater shrimp *Palaemonetes argentinus* from La Plata, Argentina.** Sergio Martorelli, Paula Marcotegui. Centro de Estudios Parasitológicos y Vectores (CCT-La Plata-UNLP), 2 Nº 584, La Plata, Buenos Aires, Argentina

M-04 **The release and establishment of microsporidia for the biological control of *Lymantria dispar* L. in Bulgaria - results of a long-term monitoring.** Andreas Linde[1] and Daniela Pilarska[2]. [1]Hochschule für nachhaltige Entwicklung Eberswalde, Dept. of Forest and Environment, Alfred-Moeller-Str. 1, 16225 Eberswalde, Germany. [2]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Blvd.Tzar Osvoboditel, 1000 Sofia, Bulgaria

Poster Session 2 Nematodes

Wednesday, 16:45 – 18:45
Room 3

N-01 **Epizootiology of the parasite *Strelkovimermis spiculatus* (Nematoda: Mermithidae) in wild mosquito populations in Argentina.** María Fernanda Achinelly and María Victoria Micieli. Centro de Estudios Parasitológicos y de Vectores, CEPAVE (CONICET-CCT La Plata-UNLP), calle 2 N° 584 (1900) Buenos Aires, Argentina

N-02 **Persistence of *Heterorhabditis amazonenis* (Rhabditida: Heterorhabditidae) in citrus field and its virulence against *Ceratitis capitata* (Diptera: Tephritidae).** Angela Canesin[1], Luís Garrigós Leite[2], Honório Roberto dos Santos[1], Marcos Gino Fernandes[1], Fabio Silber Schmidt[2], Maria de Lourdes Zamboni Costa[3] and Luis Anselmo Lopes[3]. [1]Universidade Federal da Grande Dourados, Programa de Pós-Graduação em Agronomia, Faculdade de Ciências Agrárias, CP 533, 79804-970 Dourados, MS – Brazil; [2]Instituto Biológico de Campinas, CP 70, 13092-543 Campinas, SP – Brazil; [3]Centro de Energia Nuclear na Agricultura, USP, CP 96, 13416-000 Piracicaba, SP – Brazil; *Supported by FINEP, FAPESP, CNPq, Citrovita Company and BioControle Company

N-03 **New observations of a Trematode species in the invasive slug *Arion vulgaris.*** Haukeland Solveig[1], Karin Westrum[1] and Raúl Iglesias[2]. [1]Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Høgskoleveien 7, 1432 Ås, Norway. [2]Laboratorio de Parasitología, Facultad de Biología, Edificio de Ciencias Experimentales, Campus Lagoas-Marcosende, Universidad de Vigo, 36310 Vigo, Spain

N-04 **Endemic entomopathogenic nematodes against selected fruit fly species (Diptera: Tephridae) in laboratory studies in Tanzania.** Solveig Haukeland[1], Yonna Kalinga[2], Maulid Mwatawala[2] and Amon Maerere[2]. [1]Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Høgskoleveien 7, 1432 Ås, Norway. [2]Sokoine University of Agriculture, Department of Crop Science and Production, P.O. Box 3005 Morogoro, Tanzania

N-05 ***Steinernema* spp. infection decisions change when exposed to potential hosts infected with entomopathogenic fungi.** Joe Isaac[1], Katie Mireles[1], Clint Martin[1] and Glen Stevens[1]. [1]Ferrum College; School of Natural Sciences and Mathematics, Ferrum, VA 24088 USA

N-06 **Perspectives of entomoparasitic nematode, *Steinernema feltiae* using to control main pest insects of vineyards in Georgia**. **Perspectives of entomoparasitic nematode, *Steinernema feltiae* using to control main pest insects of vineyards in Georgia.** Manana Kakhadze, Tsisia Chkhubianishvili, Mariam Chubinisvili, Iatamze Malania, Rusudan Skhirtladze, Iren Rijamadze, Matia Matiasvili, Levan Ninua NLE Georgian Agricultural University, Kanchaveli L. Institute of Plant Protection, Tbilisi, Georgia

N-07 **Control of diapausing larvae of *Cydia pomonella* in the field using two Chilean strains of entomopathogenic nematodes.** Luis Devotto[1], Loreto Merino[1], Andrés France, Irina Urtubia[1] and Daniel San Martín[2]. [1]Centro Tecnológico de Control Biológico, Instituto de Investigaciones Agropecuarias (INIA), Centro Regional de Investigación Quilamapu, Av. Vicente Méndez 515, Chillán; [2] Universidad Adventista de Chile, Facultad de Ingeniería y Negocios, Casilla 7-D, Chillán, Chile

N-08 **Selection of native isolates of entomopathogenic nematodes to control the Chilean grape weevil (*Naupactus xanthograpus*).** Irina Urtubia[1], Andrés France[1] and Paola Luppichini[2]. [1]Instituto de Investigaciones Agropecuarias, CRI Quilamapu. Vicente Méndez 515, Chillán, Chile; [2]Instituto de Investigaciones Agropecuarias, CRI La Cruz, La Cruz, Chile

N-09 **Susceptibility of eggs of *Sphenophorus levis* (Coleoptera: Curculionidae) to *Steinernema brazilense* (Rhabditida: Steinernematidae).** Lucas Detogni Simi[1,3], Luis Garrigós Leite[2], Renata Marraschi[2], Fernanda Polastre Pereira[2], Mariana Garcia Martínez-Silva[2], Ana Paula Santos-Bartels[2], Roselaine Nunes da Silva Bueno[2] and Antonio Batista Filho[2]. [1]Faculdade de Ciências Agronômicas/Universidade Estadual Paulista - Depto. de Produção Vegetal / Defesa Fitossanitária, Botucatu, São Paulo, Brazil; [2]Instituto Biológico - Laboratório de Controle Biológico, Campinas, São Paulo, Brazil

N-10 **Nematicidal activity of the *Bacillus thuringiensis* to *Meloidogyne incognita* (Nematoda: Meloidogynidae).** Diouneia Lisiane Berlitz[1,2]; Cássio de Souza da Silva[1,2]; Maximiano Corrêa Cassal[1], Rita de Cássia Santin[3], Alexandre Guimarães[3], Aida Teresinha Santos Matsumura[3] and Lidia Mariana Fiuza[1] UNISINOS, PPG in Biology, Laboratory of the Microbiology and Toxicology, Av. Unisinos, 950, CEP: 930220-00, São Leopoldo- RS; [2]CNPq/RHAE – Support; [3]ICB Bioagritec Ltda., Porto Alegre, Brazil

Poster Session 2
Viruses

Wednesday, 16:30 – 18:45
Room 3

V-20 **STU** **Mode of inheritance of resistance to a nucleopolyhedrovirus in the smaller tea tortrix, Adoxophyes honmai (Lepidopetra: Tortricidae).** Hiroto Shinomiya, Yasuhisa Kunimi and Madoka Nakai. Institute of Agriculture, Division of Bioregulation and Biointeraction. Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan

V-21 **STU** **Insect transposons: natural tools potentially involved in the evolution of baculovirus.** Núria Martínez, Mariano Nicolás Belaich, Matias Javier Garavaglia and Pablo Daniel Ghiringhelli. LIGBCM-AVI, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes. Roque Saenz Peña 352, Bernal, Pcia. Buenos Aires, Argentina (B1876BXD)

V-22 **STU** **Baculovirus diversification.** Julien Thézé[1], Jenny S. Cory[2]and Elisabeth A. Herniou[1].[1]Insect Biology Research Institute, CNRS UMR-7261, University François Rabelais, 37200 Tours, France.[2]Department of Biological Sciences, Simon Fraser University, Burnaby, V5A 1S6, British Columbia, Canada

V-23 **Baculovirus gene Ac109 is required for occluded virus production and budded virus replication.** Victoria Alfonso[1, 2], Sol Reca[2], Guillermo Maroniche[1, 2], María Gabriela López [2], Elisa Carrillo[1, 2]and Oscar Taboga[1, 2]. [1] CONICET, CABA, Argentina. [2] Instituto de Biotecnología, INTA Castelar, Hurlingham, Buenos Aires, Argentina

V-24 **An *ac34* deletion mutant of *Autographa californica nucleopolyhedrovirus* exhibits delayed late gene expression and a lack of virulence *in vivo.*** Yi Cai, Meijin Yuan, Guanghong Li and Kai Yang. State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

V-25 **STU** **Complementation of p74 KO AcMNPV using a transgenic cell line.** Cecilia Soledad Turco[1], Mariano Nicolás Belaich[1], Diego Luis Mengual Gómez[1], Alicia Sciocco-Cap[2] and Pablo Daniel Ghiringhelli[1] [1]LIGBCM-AVI (Laboratorio de Ingeniería Genética y Biología Celular y Molecular - Área Virosis de Insectos), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes (Roque Sáenz Peña 352, Bernal, Buenos Aires, Argentina. [2] IMyZA-CCVyA-INTA, Las Cabañas y los Reseros s/n, Hurlingham, Argentina

V-26 **Functional studies on the *per os* infectivity factor 3 (PIF3) of HearNPV.** Jingjiao Song, Manli Wang, Huachao Huang, Xin Luo, Fei Deng, Hualin Wang and Zhihong Hu. State Key Laboratory of Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P. R. China

V-27 ***Autographa californica* multicapsid nucleopolyhedrovirus late genes mediate Arp2/3 complex nuclear relocation during virus infection.** Jingfang Mu[1], Yun Wang[1] and Xinwen Chen[1]. [1]Wuhan Institute of Virology, Chinese Academy of Sciences. R.P. China

V-28 **Analysis of induction and suppression of apoptosis in the *Lymantira dispar* Ld652Y cells infected with nucleopolyhedroviruses.** Hayato Yamada, Koji Kitaguchi, Michihiro Kobayashi and Motoko Ikeda. Laboratory of Sericulture and Entomoresources, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

V-29 **Host insect liquefaction in infections with *Condylorrhiza vestigialis multiple nucleopolyhedrovirus*: effect of possible variation in cathepsin (*v-cath*) and chitinase (*chiA*) genes.** Marina Tagliari[1,2], Zilda M.A. Ribeiro[2] and Maria E.B. Castro[2].[1]Universidade de Brasília – UnB, [2]Embrapa Recursos Genéticos e Biotecnologia – CENARGEN, 70770-917 – Brasília, DF, Brazil

V-30 **Study of polyhedrin functional complementation among nucleopolyhedroviruses.** Santiago Haase[1], M. Gabriela López[2], Carlos Jaramillo[1], Oscar Taboga[2], Alicia Sciocco-Cap[3] and Víctor Romanowski[1]. [1]Instituto de Biotecnología y Biología Molecular, Universidad Nacional de La Plata, CONICET, Argentina. [2]Instituto de Biotecnología, CICVyA, INTA. [3]Instituto de Microbiología y Zoología Agrícola, CICVyA, INTA, Argentina

V-31 **Development of a cell line derived from High Five™ for simple titration of baculovirus.** Santiago Haase[1], M. Gabriela López[2], Carlos Jaramillo[1], Oscar Taboga[2], Alicia Sciocco-Cap[3] and Víctor Romanowski[1]. [1]Instituto de Biotecnología y Biología Molecular, Universidad Nacional de La Plata, CONICET, Argentina. [2]Instituto de Biotecnología, CICVyA, INTA. [3]Instituto de Microbiología y Zoología Agrícola, CICVyA, INTA, Argentina

V-32 **Enhanced production of Porcine circovirus type 2 capsid protein by the fusion expression with baculovirus partial polyhedron.** Jun Beom Lee, Sung Min Bae, Hee Jung Kim, Jae Bang Choi, Won Il Heo, Tae Young Shin, Yeon Ho Je[1], Byung Rae Jin[2] and Soo Dong Woo*. *Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea. [1]School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul, Korea.[2]College of Natural Resources and Life Science, Dong-A University, Busan, Korea

V-33 **Comparison of expression of haemagglutinin from H5N1 influenza virus by three different baculovirus expression systems.** Alexandra Elliott[1], Éva Nagy[2] and Peter Krell[1]. [1]Department of Molecular and Cellular Biology and [2]Dept. of Pathobiology University of Guelph, Guelph Ontario Canada N1G 2W1

V-34 **Development of an immunological technique for detecting granulovirus infection in *Tuta absoluta* larvae (Lepidoptera: Gelechiidae).** Juliana Gómez V.[1,2], Lorena Herrera C.[2] and Laura Villamizar R.[2]. [1]Universidad Nacional de Colombia. [2]Biological Control Laboratory. Biotechonology and Bioindustry Center. Colombian Corporation for Agricultural Research CORPOICA, Mosquera, Colombia

V-35 **Replication of two entomopoxviruses in CF70 cells derived from the eastern spruce budworm, *Choristoneura fumiferana.*** Srini Perera, Lillian Pavlik, Peter Krell[1] and Basil Arif. Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste Marie, Ont., Canada. [1]Department of Molecular and Cellular Biology, University of Guelph, Ont. Canada

V-36 **Biochemical characterization of the 3C-like protease from *Ectropis obliqua* virus.** Shan Ye, Congyi Zheng, Jiamin Zhang, Xi Zhou and Yuanyang Hu**.** State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei 430072 China

V-37 **Concomitant natural infections with the mermithid *Strelkovimermis spiculatus* and a mosquito iridescent virus in *Culex pipiens.*** Evangelina Muttis, Juan José García and María Victoria Micieli. Centro de Estudios Parasitológicos y de Vectores, CEPAVE (CONICET-CCT La Plata-UNLP)-, calle 2 N° 584, (1900) La Plata, Buenos Aires, Argentina

V-38 **A new insect rhabdovirus from *Culex tritaeniorhynchus* mosquitoes utilize host's nuclear splicing machinery.** Ryusei Kuwata[1], Haruhiko Isawa[1], Keita Hoshino[1], Yoshio Tsuda[1], Tohru Yanase[2], Toshinori Sasaki[1], Mutsuo Kobayashi[1], and Kyoko Sawabe[1]. [1]Department of Medical Entomology, National Institute of Infectious Diseases, Japan, and [2]Kyushu Research Station, National Institute of Animal Health, Japan

V-39 **Nucleotide sequence variations of the major structural proteins (VP15, VP19, VP26 and VP28) of white spot syndrome virus (WSSV), a pathogen of cultured *Litopenaeus vannamei* in Mexico.** Zinnia Judith Molina-Garza[1], José Luis Rosales-Encinas[2], Juan Manuel Alcocer-González[1]and Lucio Galaviz-Silva[1]. [1]Laboratorio de Patología Molecular, Centro Nacional de Sanidad Acuícola, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, Mexico. [2]Departamento de Patología Experimental, CINVESTAV-IPN, Unidad Zacatenco, DF, Mexico

Business Meetings

Evening Session 1 — **Wednesday, 18:45 – 20:30**
Microbial Control Division Business Meeting — **Auditorium 2**
Organizer. Stefan Jaronski

20:00 **Efforts of the Biopesticide Industry Alliance to Promote Microbial Agents in the U.S.** Eda Renoit (Chair of the Board of Directors, Biopesticide Industry Alliance)

Evening Session 2 — **Wednesday, 18:45 – 20:00**
Diseases of Beneficial Invertebrates Business Meeting — **Auditorium 3**
Organizer: Grant Stentiford

THURSDAY 9th

SIP 2012 Congress Registration — **Thursday, 08:00-12:00**

Morning Session 1 — **Thursday, 08:00 – 10:00**
Workshop II - Diseases of Beneficial Invertebrates Division — **Auditorium 2**

OIE-notifiable aquatic invertebrate diseases: a Latin American perspective

Organizers: Grant Stentiford, Carlos Zenobi and Sergio Martorelli

08:00 **142 Listed diseases and the global trading of aquatic crustaceans.** Grant D. Stentiford. European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, Dorset DT4 8UB, United Kingdom

08:30 **143 Presence of OIE –Notifiable viral pathogens in crustaceans from Argentina.** Sergio Martorelli Centro de Estudios Parasitológicos y Vectores (CEPAVE), CONICET-CCT La Plata, Calle 2 No. 584, La Plata 1900, Buenos Aires, Argentina

09:00 **144 First survey of notifiable viral diseases of crustaceans in wild red shrimp *Pleoticus muelleri* in the San Jorge Gulf, Argentina.** Carlos Zenobi[1], Fernando C. Raibenberg[2], C.I. Balette[2], M.A. Álvarez[1], J. De la Garza[3], D. Bottino[4], M.C. Ferreyra Armas[2], R.Balzano[2], R.Sanguinetti[2]and L.A. Romano[1]. [1]Departamento de Patología, Dirección de Laboratorio Animal, DILAB, SENASA; [2]Dirección de Acuicultura, Ministerio de Agricultura, Ganadería y Pesca. Bs As; [3]Instituto Nacional de Investigación y Desarrollo Pesquero, INIDEP, Programa de Pesquerías de Crustáceos, Mar del Plata; [4]Programa Sanitario de Organismos Acuáticos, DNSA, SENASA, Argentina

09:30 **145 Epidemiology, histopathology and ultrastructure of *Bonamia exitiosa* infected *Ostrea puelchana* and *Bonamia* sp infected O. *stentina* from San Matías Gulf, Patagonia, Argentina.** Marina A. Kroeck[1,2], Enrique M. Morsan[1,2], Erica Oehrens[1,2], Socorro Doldan[1,2], Paula Zaidman[1,2] and Manuela Calvo[3]. [1]Universidad Nacional del Comahue, Dpto. de Ciencias Marinas. San Antonio Oeste, Río Negro; [2]Instituto de Biología Marina y Pesquera "Alte. Storni". San Antonio Oeste, Río Negro; [3]Universidad Nacional del Comahue, Centro Regional Universitario Bariloche (CRUB), Río Negro, Argentina

Morning Session 2 **Thursday, 8:00 – 10:00**
Symposium X - Microbial Control Division **Auditorium 1**

Microbial Control – The Latin American Way

Organizers: Trevor Jackson and Surendra Dara

08:00 **146 Latin American successes in microbial control – a view from outside.** Trevor Jackson. AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand

08:30 **147 The use of *Bacillus thuringiensis* based biopesticide for small-scale growers in Brazil.** Fernando H. Valicente[1], Emanuel I. M. Lemos[2]and Flávio A. O. Rego[3]. [1]Embrapa Maize and Sorghum Research Center, C. P. 151, 35.701-970, Sete Lagoas, MG, Brazil. [2]Coordenador do Desenvolvimento da Agricultura Familiar-CODAF, Secretaria do Desenvolvimento Agrário, Fortaleza, Ceará, Brazil, [3]Secretaria do Desenvolvimen-to Agrário, Fortaleza, Ceará, Brazil

09:00 **148 Progress and opportunities in microbial control in the Chilean fruit industry.** Andrés France. INIA Quilamapu, Casilla 426, Chillán, Chile

09:30 **149 Microbial control of insects: A Brazilian perspective.** Daniel Ricardo Sosa-Gómez[1], Marcos Rodrigues de Faria[2], Bráulio Santos[3], José Eduardo Marcondes de Almeida[4]and Luís Garrigós Leite[4] [1]Embrapa Soja, Cx. P. 231, Londrina, PR, Brazil. E-mail: drsg@cnpso.embrapa.br, [2]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil; [3]Centro Politécnico, Cx. P. 19031, Universidade Federal do Paraná, CEP: 81531-980, Curitiba, PR, Brazil;[4] Instituto Biológico, Avenida Conselheiro Rodrigues Alves 1.252, CEP 04014-002, São Paulo, SP, Brazil

10:00 – 10:25 **BREAK**

SIP Annual Business Meeting

Thursday, 10:30– 12:30
Auditorium 1

Presentation during business meeting:

150 The three Gs: Personal reminiscences of invertebrate cell culture pioneers: Goldschmidt, Gao, and Grace. Karl Maramorosch (Entomology Department, Rutgers-State University of New Jersey, USA)

12:30 -13:45 **LUNCH**

Lunch **Thursday, 12:30 – 14:00**
Students and Postdocs Affairs Commite Panel Session **Auditorium 4**

Job opportunities for 21st Century Scientists

Organizer: Patricia Stock

Aaron Gassmann- U. Iowa, USA
Glen Stevens, U. Florida, USA
Eda Reinot, Becker-Underwood
Nicolas Pedrini, CONICET, Argentina
Clara Rubistein, Monsanto, Argentina

Afternoon Session 1 **Thursday, 14:00 – 16:00**
Workshop III – Bacteria and DBI Divisions **Auditorium 2**

Use of RNAi to control insects or diseases of insects

Organizers: William Moar and Ricardo Salvador

14:00 **151 Why is it untrue that killing the messenger doesn't solve the problem?** Esteban Hopp. Instituto de Biotecnología, INTA Castelar, CC25, 1712 Castelar, Argentina

14:15 **152 RNAi products platform for invertebrates' health and targeted pest control.** Eyal Ben-Chanoch. Beeologics, Inc., USA

14:45 **153 Design and evaluation of a strategy to control the cotton weevil, based on dsRNAs ingestion that induce gene silencing.** Ricardo Salvador[1,2], Natalia Almasia[2], José Niz[1], Marcelo Berretta[1], Cecilia Vazquez-Rovere[2], Alicia Sciocco-Cap[1] and Esteban Hopp[2]. [1]Instituto de Microbiología y Zoología Agrícola (CICV y A - INTA), Buenos Aires; [2] Instituto de Biotecnología (CICV y A - INTA), Buenos Aires, Argentina

15:00 **154 The mode of action of dsRNA for control of western corn rootworm (*Diabrotica virgifera virgifera*) larvae.** Gerrit Segers, Parthasarathy Ramaseshadri, Ron Flannagan, William Moar and Renata Bolognesi.Monsanto Company, 700 Chesterfield Pkwy W, Chesterfield, MO 63017, USA

15:30 **155 Pyramiding dsRNA with Bt to control corn rootworm.** William Moar, Tom Clark, Graham Head, Gerrit Segers, Renata Bolognesi, and Ron Flannagan. Monsanto Company, 800 North Lindbergh, Creve Coeur, MO 63167, USA

Afternoon Session 3 **Thursday, 14:00 – 15:15**
Contributed Papers **Auditorium 3**

Microbial Control 3

Chair: Manoel Victor Franco Lemos

14:00 **156 First comparative transcriptomic analysis of wild adult male and female *Lutzomyia longipalpis*, vector of visceral leishmaniasis.** Christina Beryl McCarthy[1, 2] and Luis Aníbal Diambra[1]. [1]Centro Regional de Estudios Genómicos, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Buenos Aires, Argentina; [2]Departamento de Informática y Tecnología, Universidad Nacional del Noroeste de la Provincia de Buenos Aires, Buenos Aires, Argentina

14:15 **157 Build up of pathogens within outbreak populations of native insect populations in modified land in New Zealand** Sean D.G. Marshall[1], Richard J. Townsend[1], Andreas Leclerque[2], Regina G. Kleespies[2], Jessica E. Dunbar[3], Tracey L. Nelson[1]and Trevor A. Jackson[1]. [1]AgResearch Limited, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand; [2] Federal Research Centre for Cultivated Plants, Julius Kühn-Institut, Institute for Biological Control, Heinrichstraße 243, 64287 Darmstadt, Germany; [3]Landcorp Farming Ltd, 220 Wilsons Lead Road, RD2 Westport 7892, New Zealand

14:30 **158 Expression of *Bacillus thuringiensis* toxin Cry1Ia7 in Pseudomonas fluorescens confers protection against UV radiation.** Iñigo Ruiz de Escudero[1,2], Aaron C. Asensio[1], Ainara Nepote-Górriz[2], Delia Muñoz[3] and Primitivo Caballero[1,2]. [1]Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadía, 31192 Mutilva Baja, Navarra, Spain**;** [2]Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, 31006 Pamplona, Spain

14:45 **159 A Novel formulation of biopesticide.** Munevver Muge Yazici[1], Gulengul Duman[2] and Fikrettin Sahin[1,*] [1]Yeditepe University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, 34755 Kayisdagi-Istanbul, Turke; [2]Faculty of Pharmacy, Yeditepe University, 34755, Istanbul, Turkey

15:00 **160 Use of microbial insecticides for the control of filarial vector, *Culex quinquefasciatu.s*** Kadarkarai Murugan. Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore-641 046-India

Afternoon Session 4 **Thursday, 14:00 – 15:45**
Contributed Papers **Auditorium 4**

Viruses 5

Functional Genomics II
Chairs: David Theilmann and Deng Fei

14:00 **161 Analysis of IE0 and IE1 transactivation of *Autographa californica* multiple nucleopolyhedrovirus early promoters.** Nadia R. Sokal[1], Yingchao Nie[2], Leslie G. Willis[2], Junya Yamagishi[3], Gary W. Blissard[3], Mark Rheault[1] and David A. Theilmann[1,2]. [1]Dept. of Biology, University of British Columbia Okanagan, 3333 University Way, Kelowna, BC, V1V 1V7, [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Box 5000, Summerland, B.C., Canada V0H 1Z0. [3]Boyce Thompson Institute at Cornell University, Tower Road, Ithaca, New York USA 14853-1801

14:15 **162 Stability regulation of baculovirus-encoded N-WASP homologous protein P78/83.** Shili Han[1], Yun Wang[2], Xinwen Chen[2]. [1]College of Life Science, Central China Normal University; [2]Wuhan Institute of Virology, Chinese Academy of Sciences

14:30 **163 Deletion of orf114 of AcMNPV diminishes its per os infectivity by reducing the numbers of ODVs in occlusion bodies.** Wenqiang Wei, Yin Zhou and Xiulian Sun. Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

14:45 **164 A Group II alphabaculovirus core gene, MacoNPV-A *pif-5* (*odv-e56*), cannot repair the essential *per os* infectivity function of an AcMNPV-*pif5* knockout virus in *Trichoplusia ni* larvae.** Ajay B. Maghodia[1], Minggang Fang[2], David A. Theilmann[2] and Martin A. Erlandson[1]. [1]Saskatoon Research Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2; and [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Box 5000, Summerland, British Columbia, Canada V0H 1Z0

15:00 **165 Characterization of novel components of the baculovirus *per os* infectivity factor (PIF) complex.** Ke Peng[1], Jan W.M. van Lent[1], Sjef Boeren[2], Minggang Fang[3], David A. Theilmann[3], Martin A. Erlandson[4], Just M. Vlak[1]and Monique M. van Oers[1]. [1]Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands; [2] Laboratory of Biochemistry, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, the Netherlands; [3] Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada; [4] Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatchewan, Canada

15:15 **166 HSP70 induction during baculovirus infection.** Jonathan Breitenbach and Holly Popham. USDA-ARS Biological Control of Insects Research Laboratory, Columbia, MO, USA

15:30 **167 Identification and characterization of the initiator caspase SfDronc in *Spodoptera frugiperda* and its role in apoptosis induced by *Autographa californica* M nucleopolyhedrovirus.** Ning Huang[1], Srgjan Civciristov[2], Christine Hawkins[2] and Rollie J. Clem[1]. [1]Division of Biology, Kansas State University, Manhattan, KS USA; [2]Department of Biochemistry, La Trobe University, Bundoora 3086, Victoria, Australia

15:45 **168 Characterization of the interaction between the AcMNPV sulfhydryl oxidase Ac92 and the *Spodoptera frugiperda* P53 protein.** [1]Wenbi Wu, Ning Huang, [1]Rollie J. Clem, [2]George F. Rohrmann and [1]A. Lorena Passarelli. [1]Division of Biology, Kansas State University, Manhattan, KS 66506; [2]Department of Microbiology, Oregon State University, Corvallis, OR 97331

16:00 **169 Gut microbiota promotes baculovirus pathogenesis.** Agata K. Jakubowska[1], Heiko Vogel[2], Juan Ferré[1] and Salvador Herrero[1]. [1]Department of Genetics, University of Valencia, Dr Moliner 50, 46100 Burjassot, Spain. [2]Department of Entomology, Max Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, 07745 Jena, Germany

20:30 – 2:00	**SIP BANQUET**	Palacio San Miguel

We hope to see you in 2013 in Pittsburgh!

ABSTRACTS

SIP 2012

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STU **indicates papers being judged for graduate student presentation awards.**

126 **indicates abstract number for ORAL presentations**

B-15 **indicates abstract number for POSTER presentations**

MONDAY 6th

Plenary Symposium Monday, 10:30-12:30

Microbial Control in Public Health and Veterinary Medicine: Reality and Expectations

Plenary Session Monday, 10:30 **1**

Entomopathogenic fungi can change the paradigm to control blood-sucking insects: the case of Chagas disease vectors

Nicolás Pedrini and M. Patricia Juárez

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Control of disease vectors is mostly based on domiciliary spraying with residual pyrethroid formulations. The rising development of pyrethroid-resistant vector populations that co-habit with humans, i.e. mosquitoes, flies and kissing bugs, urged searching new control alternatives. Among them, the use of entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* have been successfully established, currently representing the sole alternative against pyrethroid-resistant insects. This presentation will focus on the potential use of an "attraction-infection" trap to control Chagas disease vectors, combining a powder formulation of *B. bassiana* with insect pheromones. The efficacy of this methodology in field trials in the Argentina-Bolivia border will be summarized. Our results show the relevance of the horizontal transmission process to the overall performance of the fungal formulation. A mathematical model is being tested to better understand the population dynamics of fungus-infected bugs, predicting the suitability of this methodology to help controlling the spread of pyrethroid-resistant bugs.

Plenary Session Monday, 11:00 **2**

Use of entomopathogenic bacteria in biological control of mosquitoes and simuliids in Brazil: a critical overview

Carlos José Pereira da Cunha Araújo-Coutinho

Laboratório de Entomologia Médica, Superintendência de Controle de Endemias, São Paulo, Brazil (cjpcacoutinho@gmail.com)

Microbial control of insect vector populations has advantages over chemical control due to host specificity which makes them more environmentally friendly. Bacteria used in biological control that have successfully suppressed mosquito larvae populations are *Bacillus thuringiensis* serovar *israelensis* (*Bti*) and *Lysinibacillus sphaericus*. In the 1980s, began in Brazil the use of bacteria for biological control of mosquitoes and blackflies, since a number of control programs were established, some with very satisfactory results, while some others failed. This presentation will approach a critical overview of the results of 20 years use of entomopathogenic bacteria for biological control of insect vectors in Brazil.

Plenary Session Monday, 11:30 **3**

A bacterium against dengue: our challenge

Luciano A. Moreira

Centro de Pesquisas René Rachou, FIOCRUZ-MG, Brazil.

Dengue has reemerged as a major public health problem in Brazil, with more than 3 million reported cases between 2000-2005, representing more than 70% of all cases reported in the Americas, and 61% of all cases reported to the WHO globally. Current control methods rely on insecticides for mosquito control and because of that, resistance against commonly used chemicals is increasingly widespread. Our project involves the use of a naturally occurring bacterium called *Wolbachia* as a novel biological control agent. *Wolbachia* manipulates the reproduction of their host in order to be vertically transmitted from the mother to offspring. This bacterium is believed to be present in up to 70% of all insect species worldwide but it has never been found in the *Aedes aegypti* mosquito (dengue vector). When stably introduced into *Aedes aegypti, Wolbachia* was able to block dengue virus transmission by these mosquitoes, constituting a great potential for control of dengue disease. Currently field tests are been carried out in Australia, where *Wolbachia* infected mosquitoes were able to invade local populations of *A. aegypti*. Next, the strategy will be applied in dengue endemic countries, like Brazil, to test whether it might be used as a sustainable dengue control strategy.

Plenary Session Monday, 12:00 **4**

First and second generation paratransgenesis: tools for the control of global vector-borne diseases

Ravi V. Durvasula

The Center for Global Health, Dept of Internal Medicine, University of New Mexico School of Medicine, Albuquerque, NM USA (ravi.durvasula@va.gov)

Despite great advances in public health, insect-transmitted infectious diseases remain a leading cause of morbidity and mortality. Currently, the best methods for control of insect-borne diseases involve the use of chemical pesticides. Such campaigns may yield spectacular results, yet long-term efficacy remains a problem. Environmental toxicity, adverse effects on human health, emergence of insect resistance and the prohibitive cost limit the use of many pesticides. Therefore, the elimination of insect pests is neither practical nor probable. Evolving methods for control of vector-borne diseases rely on modification of insects. Paratransgenesis is a "Trojan Horse" approach to control of disease transmission. It employs the interactions between disease-transmitting vectors, bacterial commensals of the vectors and transmitted pathogens. Commensal bacteria are isolated and genetically transformed *in vitro* to export molecules that interfere with pathogen transmission. The genetically altered bacteria are introduced into the host vector where expression of engineered molecules affects the host's ability to transmit the pathogen. This approach attempts to decrease pathogen transmission and employs, as a gene delivery mechanism, bacterial flora native to the host vector. The model system for paratransgenic control involves the vectors of Chagas disease, a disease of Central and South America. Paratransgenic methods are under development for control of sand fly-mediated leishmaniasis and Pierce's Disease, an agricultural disease of grapes and citrus crops that is transmitted by Glassy Winged Sharpshooters. Finally, second generation paratransgenic systems are under development that employ advanced nano-materials to achieve better targeting of recombinant molecules with minimal environmental impact of transgene release. This session will provide an overview of activities in the Paratransgenesis Laboratory of The Center for Global Health, Albuquerque, USA.

Symposium I - Virus Division Monday, 14:00-16:00

Viral biocontrol

Symposium I Monday, 14:00 **5**

Dr. Flavio Moscardi and his relevant contribution to viral biocontrol in South America

Marlinda L. Souza

Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Av. W5 Norte final, Brasília, DF, Brazil, CEP 70.770-900. (marlinda.souza@embrapa.br)

Dr. Flavio Moscardi was graduated in Agricultural Sciences at ESALQ/University of São Paulo (Brazil) in 1973. He got the Master and PhD degrees at the University of Florida (USA), from 1975 to 1979, developing studies on the biology and ecology of the velvetbean caterpillar and on its pathogenic virus, the *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV). Back to Brazil he started to work as a scientist at Embrapa, the Brazilian Agricultural Research Corporation, where he began to implement a velvetbean caterpillar control program with baculovirus. This was the most successful program worldwide with a virus pesticide, starting from early eighties and lasting for more than thirty years. The virus was also used in Argentina, Colombia, Bolivia, Paraguay and Mexico. The maximum peak of AgMNPV use occurred in the season 2003/2004, when approximately two million ha of soybean were applied in Brazil. Afterwards, the virus use declined sharply due to changes in farmers' procedures to control pests in soybean. Currently about 300,000 ha are being treated yearly. Dr. Moscardi had been also an effective consultant in countries as Argentina, Paraguay, Uruguay, Nicaragua, Indonesia, Philippines, North Korea, Tanzania and India. During his carrier, he published more than 200 publications and advised many graduated and undergraduated students. Due to his relevant contributions, Dr. Moscardi was elected to the Brazilian Academy of Sciences in 2003 and received 25 prizes/titles, such as the Young Scientist First Prize from CNPq (1983), and the Commend of the National Order of Scientific Merit from the President of Brazil (2002).

Symposium I Monday, 14:30 **6**

Baculovirus: research and commercialization in Colombia

Laura Villamizar R.

Biological Control Laboratory. Biotechnology and Bioindustry Center. Colombian Corporation for Agricultural Research (CORPOICA). Mosquera, Colombia. (lvillamizar@corpoica.org.co)

Baculoviridae family is the most numerous and extensively studied of all entomopathogenic viruses. In Colombia only two viruses of this family have been registered and commercially exploited, one nucleopolyhedrovirus of *Trichoplusia ni* denominated "Trichovirus", that was used between 1970 and 1973 for controlling this insect in cotton crops, with such success that in a few years the pest disappeared almost completely. The other is a granulovirus of the potato moth *Phthorimaea operculella* denoted "Baculovirus Corpoica" which is currently commercialized for controlling *Tecia solanivora* in stored potato tubers with more than 80% of efficacy. Recently the interest in developing, registering and marketing biopesticides based on baculovirus has increased considering its high pathogenicity and virulence, specificity and shelf life. In this sense several researches have been directed to collect and characterize new native isolations from *Tecia solanivora* and *Spodoptera frugiperda*. In these works an interesting genetic diversity in Colombian viruses has been observed. Then, an emulsifyble concentrated was developed with one granulovirus of *T. solanivora* and a microencapsulated wettable powder with one nucleopolyhedrovirus of *S. frugiperda*, both products including efficient protection against ultraviolet radiation and with efficacies higher than 80% in potato and maize crops respectively. The manufacture process of both biopesticides has been scaled up to a pilot plant level and registration process is now in course. In a nearby future, Colombian farmers will have new biopesticides based on baculoviruses for the management of two limiting pest in agricultural production.

Symposium I Monday, 15:00 **7**

Application of slow-killing granuloviruses to control leaf-rollers in tea fields in Japan

Madoka Nakai

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The size of the Asian microbial control market is increasing, and comprises *Bacillus thuringiensis*, fungi and viruses. China is the biggest market, followed by India and Japan. Two microbial control agents based on baculoviruses are currently registered in the Japanese market: a mixture of granuloviruses (GVs) to control leaf-rollers (*Adoxophyes honmai* and *Homona magnanima*; Lepidoptera, Tortricidae) in tea fields, and a nucleopolyhedrovirus (NPV) to control armyworm (*Spodoptera litura*; Lepidoptera, Noctuidae). Both agents are required to control these pests, which have acquired resistance against chemical pesticides. The GVs for *A. honmai* and *H. magnanima* are well adapted to control leaf-rollers in tea systems, and are also advantageous for virus production using living insects. A notable feature of this system is that a single virus application can suppress the leaf-rollers through a whole year, during which four generations of the pest occur. This is because the virus kills the host slowly and thus enables progeny virus to transmit itself to young insects in the next host generation. The prolonged survival period of first-generation GV-infected hosts has no detrimental economic impact, because the first tea harvest in May is the most valuable and leaf-roller populations become abundant only after this first harvest. A natural field survey of *A. honmai* populations in Ibaraki prefecture revealed that an entomopoxvirus was highly prevalent, followed by *A. honmai* NPV. These viruses also kill the host more slowly than typical NPVs such as *Autographa californica* NPV. Slow-killing viruses may have adapted to leaf-roller pests of evergreen and perennial tea plants.

Symposium I Monday, 15:30 **8**

The use of *Cydia pomonella* granulovirus in organic and integrated pest management

Johannes A. Jehle

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Since its first description in 1964 the *Cydia pomonella* granulovirus (CpGV-M) has become an important biocontrol agent of codling moth in both organic and integrated production of apples, pears and walnut. Today, CpGV is the active ingredient of one of the economically most successful commercial baculovirus products that is used in nearly all pome fruit growing areas all over the world. It is highly effective, environmentally friendly and can be ideally combined with chemical insecticides. Therefore, it plays an increasingly important role in integrated plant protection strategies, esp. as it helps to provide long-term population control and to reduce residues of chemical insecticides on the fruit. However, this success was threatened in 2005, when first reports on codling moth populations with a dramatically decreased susceptibility to CpGV products became available. Since then, CpGV resistance has been noted in more than 40 orchards in different European countries. Though the resistance mechanism is still not fully elucidated, it was shown that a dominant, Z-linked inheritance of the resistance allele has contributed to the rapid emergence of CpGV resistance. Fortunately, other than CpGV-M isolates are able to overcome CpGV resistance and are now registered and used in Europe. The diversity of CpGV isolates provides the necessary tools to improve codling moth control and may also allow controlling other tortricids, such as *C. molesta*. In the light of more than 20 years of experience with commercial CpGV application strategies of resistance management are highly recommended.

Workshop I Monday, 14:00- 15:00
Microsporidia and DBI Divisions

Host range of Microsporidia

Workshop I Monday, 14:00 **9**

Host specificity and effects of microsporidia that infect natural enemies used for biological pest control

Susan Bjornson

Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, Nova Scotia, Canada. (susan.bjornson@smu.ca)

Microsporidia are known to infect several natural enemies that are used for biological pest control, including endoparasitic wasps (*Cotesia*, *Muscidifurax*), aphidophagous lady beetles (*Adalia, Hippodamia)* and predatory mites (*Phytoseiulus, Metaseiulus*). Microsporidia often cause chronic, debilitating disease, which has a detrimental impact on host fitness; however, in some cases, natural enemies may be affected by microsporidia in unpredictable (and less measurable) ways. Past identifications of microsporidia in natural enemies were often based on light microscopic observations of pathogen development and tissue pathology. Much consideration was given to the particular host species that was infected. This resulted in the description of several new microsporidian species from light microscopic observation of spores from related hosts. However, recent work has shown that a particular natural enemy may be infected with more than one species of microsporidia under laboratory conditions. This factor raises questions regarding the true identity of microsporidia in natural enemies, particularly when conclusions are based on light microscopic observations of microsporidian spores that appear similar in shape and size. To further confound the issue, the geographical distribution of a natural enemy that is susceptible to a particular microsporidium may overlap with other related host species that are also susceptible. This provides the potential for pathogen dissemination among several related host species once microsporidia-infected natural enemies are released in the local environment. Microsporidian spores are often transmitted transovarially. Cannibalism is common among lady beetles and other generalist predators and is a good means of pathogen dissemination. Other factors (the presence of other pathogens, endoparasitic wasps, and variations in environmental conditions) can also play a role in pathogen dispersal.

Contributed Papers Monday, 15:00-16:15

Microsporidia 1

Contributed Papers Microsporidia 1 Monday 15:00 **10**

Pathology and effects of a new microsporidium from the green lacewing, *Chrysopa carnea* used for biological pest control

Susan Bjornson and Thomas Steele

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Green lacewings, *Chrysopa carnea* and other related species, are generalist predators that are often used for controlling various agricultural pests, including aphids, mealybugs, scales, spider mites, thrips, and whiteflies. During a routine examination of green lacewing larvae, a previously unreported microsporidium was detected in specimens obtained from a commercial insectary. Infection of larvae resulted in discoloration and mortality. Prior to death, infected larvae often turned black, a stark contrast to the normal light tan colouration of uninfected larvae. Infected adults, whether they were able to emerge or not, had malformed wings. Some infected adults died before they could eclose, often with their pupal cases attached to their body. Molecular sequencing revealed that the pathogen likely represents a new species, being 96% similar to *Nosema granulosis*; a transovarially transmitted feminizing microsporidium that infects the amphipod crustacean, *Gammarus duebeni*.

Contributed Papers Microsporidia 1 Monday 15:15 **11**

Ultrastructure and pathology of a novel microsporidian pathogen in the two-spotted ladybeetle, *Adalia bipunctata* L.

Thomas Steele and Susan Bjørnson

Biology Department, Saint Mary's University, 923 Robie Street, Halifax, NS B3H 3C3 Canada. (steelem4@hotmail.com)

The two-spotted lady beetle, *Adalia bipunctata* L., is an important natural enemy that is used for pest control in North America and Europe. *A. bipunctata* are known to host a wide variety of symbionts, including microsporidia. Microsporidia are common pathogens of other lady beetle species and often cause chronic, debilitating disease by reducing host fitness. Recently, an unidentified microsporidium was isolated from two-spotted lady beetles in Nova Scotia, Canada. Molecular characterization of the microsporidian genome has revealed that it is a novel species. The objective of this study is to describe the ultrastructure and associated tissue pathology of the microsporidian pathogen using transmission electron microscopy (TEM) and light microscopy, respectively. Healthy and microsporidia-infected lady beetles were reared under controlled conditions in the laboratory. Larval and adult tissues were embedded in resin for examination by TEM. Micrographs were used to describe pathogen ultrastructure of both vegetative stages and mature spores. To determine tissue pathology, both uninfected and microsporidia-infected *A. bipunctata* adult and larval tissues were embedded in paraffin for examination by light microscopy. Information gained from this study will form the basis of a formal description of the pathogen and will provide a framework for future studies regarding host-pathogen interrelationships.

Contributed Papers Microsporidia 1 Monday 15:30 **12**

New species of spore-forming pathogens (nephridiophagids) in Malpighian tubules of insects

Renate Radek, Daniel Wellmanns and Anja Wolf

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Malpighian tubules of insects may be colonized by a variety of unicellular pathogens such as amoeba, flagellates, gregarines, coccidia, microsporidia and nephridiophagids. Nephridiophagids have been mainly reported from cockroaches and beetles. Their life cycle comprises multinucleate plasmodial stages that may divide into small uni- or oligonucleate merozoite-like stages or transform into sporogenic plasmodia (pansporoblasts). Spores are formed endogenously. Mature sporogenic plasmodia contain numerous flattened, oval spores with one nucleus and residual vegetative nuclei in the mother cytoplasm. Generally, different species are differentiated by the number of spores per pansporoblast, size and form of the mature spores, and extra- or intracellular location. Seemingly, also the identity of the host contributes a character for species determination. We discovered two new species of *Nephridiophaga* in cockroaches, i.e. *N. archimandrita* from *Archimandrita tessellata* and *N. lucihormetica* from *Lucihormetica verrucosa* (Radek et al., 2011). All vegetative and sporogenic stages of these species occur in the lumen of the Malpighian tubules. The spores of *N. lucihormetica* are slightly longer than the spores of *N. archimandrita* and their pansporoblasts contain fewer spores. The systematic affiliation of the Nephridiophagidae (syn. Coelosporidiidae) is not yet fully elucidated.

Radek, R., Wellmanns, D., Wolf, A. (2011): Two new species of *Nephridiophaga* (Zygomycota) in the Malpighian tubules of cockroaches. Parasitol. Res. 109: 473–485.

Contributed Papers Microsporidia 1 Monday 15:45 **13**

Genomes of microsporidia in mosquitoes: status and preliminary findings

James J. Becnel and Neil Sanscrainte

Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL 32608, USA. (James.Becnel@ars.usda.gov)

The status and preliminary findings for full genome sequencing of three species of microsporidia with mosquitoes as type hosts will be presented. *Vavraia culicis*, the type species of the genus *Vavraia*, was originally described from *Culex pipiens*. Type material was not available and therefore *Vavraia culicis floridensis* isolated from *Aedes albopictus* in Florida was used for sequencing. *V. culicis* has a broad mosquito host range, is infectious for several species of Lepidoptera and characterized by having only uninucleate stages and produces uninucleate spores in multisporous sporophorous vesicles. *Anncaliia (=Nosema) algerae*, originally described from *Anopheles stephensi,* has one of the broadest host ranges of any species of microsporidia including many invertebrate and vertebrate hosts. *A. algerae* is characterized by binucleate (diplokaryotic) stages and is disporous producing individual diplokaryotic spores without a sporophorous vesicle. *Edhazardia aedis* is the type species for the genus and has a limited host range in mosquitoes and can only complete its life cycle in *Ae.aegypti*. *E. aedis* is polymorphic, producing 4 distinctive spore types. It is transmitted both horizontally and vertically and requires 2 generations of the mosquito host to complete the life cycle. Genome sequencing for *E. aedis* and *V. culicis floridensis* are completed. *V. culicis floridensis* has a genome size of approximately 6.1Mb while *E. aedis* is significantly larger at approximately 51Mb. *A. algerae* sequencing is underway. Preliminary genome features will be presented.

Contributed Papers Microsporidia 1 Monday 16:00 **14**

Plastic parasites: extreme dimorphism in a microsporidium infecting the musculature of crabs

G.D. Stentiford*, K.B. Bateman, S.W. Feist, E. Chambers and D.M. Stone

European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset DT4 8UB, United Kingdom. *(grant.stentiford@cefas.co.uk)

The current taxonomy of the Phylum Microsporidia is being increasingly challenged by the use of nucleic acid-based approaches to phylogeny. The contradiction between morphology-based taxonomy and that based upon phylogenetics is problematic when attempting to describe novel taxa. A serendipitous discovery by our laboratory has provided a key example of this issue by demonstrating the potential for extreme dimorphism in a microsporidian parasite infecting a single cell type of a single species of marine crab (the common European shore crab *Carcinus maenas*). Furthermore, the discovery has necessitated a complete re-description of the parasite to encompass these previously undescribed life stages. The parasite appears to alternate between a primarily diplokaryotic lineage which culminates in unusual monokaryotic needle-like spores (*Nadelspora*-type), and a primarily monokaryotic lineage that culminates in monokaryotic spores with pronounced surface projections (*Ameson*-type). Both lineages occur in direct contact with the cytoplasm of host muscle cells and can exist simultaneously in the same cell. Chance inclusion of the microsporidian parasites *Nadelspora canceri* (from the marine crab *Cancer magister*) and *Ameson michaelis* (from the marine crab *Callinectes sapidus*) in previously published phylogenetic assemblages based upon partial sequences of the SSU rRNA gene have demonstrated (though have not discussed) a very close relationship between these two parasite genera, despite the fact that their described spore morphology and developmental cycle is very different, and in different hosts. Analysis of the SSU rRNA gene in infected *C. maenas* from this study appears to confirm genetic synonymy of the two spore types. The discovery reported here provides evidence that the morphologically divergent genera *Ameson* and *Nadelspora,* both previously described infecting the musculature of marine crabs, are potentially life cycle variants of the same taxon. Furthermore, they appear to form a clade with other morphologically diverse but phylogenetically and ecologically similar muscle-infecting microsporidians from marine crustacean hosts. In terms of taxonomy, the microsporidian parasite *Ameson pulvis* Perez 1905, infecting *C. maenas*, is shown to possess a previously undescribed lineage of life stages which culminate in the formation of needle-like spores.

Contributed Papers Monday, 14:00-15:30

Bacteria 1

Contributed Papers Bacteria 1 Monday 14:00 **15 STU**

Entomopathogenic nematodes as disseminating agents for *Yersinia pseudotuberculosis*: A laboratory model.

Samuel Gengler[1,2], Anne Laudisoit[3] and Pierre Wattiau[1]

[1]Veterinary & Agrochemical Research Centre, Brussels, Belgium; [2]Institut of life sciences, Université catholique de Louvain-la-Neuve (UCL), Belgium; [3]School of Biological Sciences, University of Liverpool, United Kingdom (samuel.gengler@coda-cerva.be)

The existence of biological micro-reservoirs for pathogenic microorganisms explaining the long-term survival of these pathogens in the environment has long been speculated. The capacity of soil invertebrates to act as intermediary hosts was the starting question of our study and entomopathogenic nematodes (EPNs) were investigated in this respect. EPNs are able to invade, kill and feed on insect cadavers thanks to a species-specific symbiotic bacterium belonging to the family Enterobacteriaceae (*Xenorhabdus* or *Photorhabdus* spp). The symbiont provides a number of biological functions that are essential for its EPN host including the production of entomotoxins, of enzymes able to degrade the insect constitutive macromolecules and of bacterial toxins able to prevent the growth of competitors in the insect cadaver. We wondered whether notorious mammalian pathogens taxonomically related to *Xenorhabdus* were able to substitute for or to "hack" the symbiotic relationship associating *Xenorhabdus* and *Steinernema* EPNs. To deal with this question, we studied a dynamic laboratory model consisting in *Galleria mellonella* insect larvae, an African *Steinernema* EPN species with its natural *Xenorhabdus* symbiont and *Yersinia pseudotuberculosis*, the etiologic agent of a gastro-intestinal disease affecting animals and humans, which was injected in the haemocoel of the insect larvae prior to infection with EPNs. Our results show that the number of *Y. pseudotuberculosis* CFUs retrieved from EPNs after 7 consecutive infection cycles - lasting for about 2 months - is comparable to the initial inoculum. In other words, the laboratory model under study demonstrates the capacity of EPNs to act as a micro-reservoir ensuring maintenance and dissemination of the pathogen. We also show that not all *Enterobacteriaceae* behave like *Y. pseudotuberculosis* inside EPNs. Genetic determinants that allowed *Y. pseudotuberculosis* to maintain inside EPNs are currently under study. The potential implication of the recently discovered type 6 secretion system components as well as that of other genes shared by both *Yersinia* and *Xenorhabdus spp*. is systematically investigated. If they turn out to have an environmental significance, these findings may reveal an unexpected biotic reservoir explaining the long-term persistence and dissemination of pathogenic bacteria in the environment.

Contributed Papers Bacteria 1 Monday 14:15 **16**

Insecticidal activity of plant root-associated Pseudomonads: host-specific expression of the fit insect toxin

Peter Kupferschmied [1], Maria Péchy-Tarr [1], Beat Ruffner [2], Monika Maurhofer [2] and Christoph Keel [1]

[1]Department of Fundamental Microbiology (DMF), University of Lausanne, Switzerland; [2]Plant Pathology, Institute of Integrative Biology (IBZ), ETH Zurich, Switzerland. (peter.kupferschmied@unil.ch)

Some fluorescent pseudomonads are well-known to effectively colonize plant roots and to protect them against fungal pathogens mainly by producing antimicrobial metabolites. We discovered that some of these bacteria also exhibit potent toxicity towards certain insects. The observed insecticidal activity relies on the production of a novel large protein toxin termed Fit and additional yet unidentified bacterial factors. The Fit toxin gene is part of a virulence cassette coding for regulators and a type I secretion system. We use our model biocontrol agent *Pseudomonas fluorescens* CHA0 to study the

molecular basis and regulation of the insecticidal activity of these bacteria. Using fluorescent reporter fusions and epifluorescence microscopy, we recently were able to visualize an induced toxin expression in strain CHA0 during systemic infection of insect larvae. In contrast, toxin production was not observed on plant roots and in several common culture media. A distinct growth medium mimicking the insect haemolymph has allowed us to study the toxin induction *in vitro* in a controllable and reproducible manner. Thereby, we got interesting insights into the roles of the local regulators in the induction of Fit toxin expression within insects. By sensing the chemical composition within the insect host, a hybrid histidine kinase seems to play a key role in the tight regulation of the toxin production. Our results suggest that *P. fluorescens* CHA0, known to be a plant root-associated bacterium, has the astonishing capability of recognizing the insect host and specifically expressing its insect toxin only during an infection of insects.

Contributed Papers Bacteria 1 Monday 14:30 **17**

The relationships between Bt`s toxic activity and population distribution

Changlong Shu, Chunge Zhang, Lian Xu, Dafang Huang, Fuping Song and Jie Zhang

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The previous reported experiment indicated that *Bacillus thuringiensis* (Bt) reproduction better in a Bt-infected cadaver than in soil. Also, while Bt reproduced in an insect cadaver, the toxin plasmids can transfer to Bc-like strain by conjunctions. Darwin's theory of evolution states that individuals with characteristics which increase their probability of survival will have more opportunities to reproduce and their offspring will also benefit from the heritable, advantageous character. So over time these variants will spread through the population. As the Cry toxins importance in Bt infection and reproduction, the Cry toxins with higher or broader toxic will be an advantageous character for the Bt spread through the population. In this report, we investigated the Bt strains diversity and distribution by a molecular mark developed from Cry toxin genes. By comparing the strains toxic activity with the data of Bt strains diversity and distribution, the relationship between Bt strain's toxic activity and population distribution also investigated.

Contributed Papers Bacteria 1 Monday 14:45 **18 STU**

Screening of *cry 1* genes in *Bacillus thuringiensis* strains against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

Arthur Augusto Gonçalves Torres[2], Rosane Bezerra da Silva[1], André Henrique Campelo Mourão[2], Thais Barros Rodrigues[1]; Camila da Silva Fernandes[2], Kátia Gisele Brasil Boregas[3] and Fernando Hercos Valicente[3]

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Proteins produced by *Bacillus thuringiensis* are widely used for pest control in agriculture. These proteins are produced by cry genes. Each or some *cry* genes show toxicity to only one insect order, whereas others present toxicity to more than one insect order. Within the techniques used in the search for new cry genes, PCR has been distinguished by its detection level, facility, practicability, and quickness. Out of a total of ninety-five Bt strains, ten were kindly provided by the USDA (United States Department of Agriculture), nine kindly provided by the Institute Pasteur (IP), eight belong to Embrapa Maize and Sorghum Bt bank. Also, 68 strains with no subspecies information (Embrapa Bt Bank) that showed larval mortality above 75% were selected from 4459 isolates previously evaluated towards *Spodoptera frugiperda* mortality, molecular characterizations were PCR based using specific *cry1* primers (*cry1A*, *cry1B*, *cry1C*, *cry1D*, *cry1E*, *cry1F*, *cry1G*, *cry1H* and *cry1I*). Three isolates did not amplify the expected fragments; all other strains showed at least one fragment and most frequent genes (84%) was *cry1D*, whereas the least frequent genes was *cry1G* (3%). Three strains (HD 29 *Bt galleriare*, 1658 and 1657) amplified 75% and 81.25% of the primers used, respectively, however, strain HD 29 *Bt galleriare* caused no mortality against *S. frugiperda*. Our results suggest no relationship between presence of *cry 1* genes and larval mortality, and make molecular characterization important for all isolates, even those that did not show any mortality of insect pests.

Contributed Papers Bacteria 1 Monday 15:00 **19**

Selection of *Bacillus thuringiensis* strains active against economically important soybean lepidopteran insects in Argentina

Diego Sauka and Graciela Benintende

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Synthetic insecticides have been effectively used worldwide, but their application has become problematic because of a range of factors, causing an increased relevance in the use of *Bacillus thuringiensis* products. Notwithstanding, in Argentina most of these kinds of products must be imported, motivating corresponding increases in costs, and leading to a growing need for developing technologies that can achieve local products based on *B. thuringiensis* to be used in insect control programs. A total of 251 *B. thuringiensis* isolates were characterized by the presence of the *cry1*, *cry2* and *cry9* genes by PCR and PCR-RFLP analysis and for the presence of crystal proteins by SDS-PAGE. Those collected from the same sample, that harbor the same *cry* gene and crystal protein profiles were considered twin strains and discarded in order to overestimate distribution frequencies. The selected *B. thuringiensis* isolates were submitted to bioassays against two economically important soybean insects; the lepidopterans *Anticarsia gemmatalis* and *Epinotia aporema*. Most of the isolates reacted with the *cry1* and *cry2* primers, but only a few strains reacted with *cry9* primers. This screening identified new *B. thuringiensis* isolates that showed high activity against the two lepidopteran species, which could be available as active ingredients of phyto-sanitary formulations, as well as their *cry* genes will be characterized and potentially used in Bt crops.

Contributed Papers Bacteria 1 Monday 15:15 **20 STU**

Characterization of naturally occurring mutations in Cry1Aa and Cry1Ac *Bacillus thuringiensis* toxins

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Bacillus thuringiensis is a spore-forming bacterium that synthesizes a parasporal crystal encoded by the *cry* genes family. Amongst many strains isolated from Lebanese soil, the strain Lip showed the highest toxicity towards lepidopteran larvae *Ephestia kuehniella* compared to the reference strain Kurstaki HD1. The dose killing 50 % of the larvae, (LD_{50}) was 33.27 and to 128.61 µg of toxin per g of flour respectively for Lip and HD1. Therefore this strain was studied for its biopesticide crystal productivity and showed 20 % higher yield than HD1. To understand the greater toxicity, the genes *cry1Aa*, *cry1Ab* and *cry1Ac*, producing the main toxins active against lepidopteran were cloned, sequenced and expressed in the acrystalliferous strain of *B. thuringiensis HD1CryB*. Compared respectively to *Cry1Aa* and *Cry1Ac* of HD1, *Cry1Aa* from *Lip*

presented the following mutations P77L, L148F, N166T and D678E while *Cry1Ac* showed the following mutations F148L and L366F. P77L is localized in the helix α2b of domain I, L148F and F148L localized in the helix α4 of domain I, N166T localized in the helix α5 of domain I and L366F in the β sheet 6 of domain II. Knowing the importance of the helices 4 and 5 of domain I in the pore formation process occurring during the toxicity process, these mutations could explain the higher toxicity of the strain Lip. At the molecular level, the interaction of these proteins with lipids and or specific receptors constructs is under study. We have selected a highly toxic Bt strain Lip and the proteins *Cry1Aa* and *Cry1Ac* are under deeper characterization.

Contributed Papers Monday, 14:00-16:00

Fungi 1

Contributed Papers Fungi 1 Monday 14:00 **21 STU**

Assessment of environmental conditions for the successful use of *Neozygites floridana*

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Neozygites floridana is an important natural enemy of spider mites. However, growers use acaricides as the main control strategy but they evolve resistance very rapidly. *N. floridana* is a good candidate for biological control of spider mites. To enable its effective use, basic information is still needed for its *in vivo* production and field use. Effect of pesticides on Brazilian and Norwegian *N. floridana* isolates were tested to be able to adapt the use of *N. floridana* into an integrated pest management (IPM) system. This study determined the optimum relative humidity (RH) and temperature for a Brazilian *N. floridana* isolate as well as the effect of photoperiod and light intensity on sporulation and germination of isolates from Norway and Brazil. RH and temperature studies were conducted through an innovative methodology that checked the leaf surface microclimate while the effect of photoperiod and light intensity on sporulation and germination of conidia was accomplished through exposure to two light intensities [40 µmol.m-2s-1 and 208 µmol.m-2s-1], three periods of exposure (24h of constant light, 12h of light preceded by 12h of dark and 24h of constant darkness) and two temperatures (18°C and 23°C). The results show that microclimatic RH within the boundary layer of a leaf is a critical factor as sporulation of *N. floridana* was only observed at >90% RH. Photoperiod of 24h and an intensity of 208 $\mu mol.m^{-2}s^{-1}$ inhibited sporulation of both isolates at both temperatures. Light intensity of 40 $\mu mol.m^{-2}s^{-1}$ had no effect on sporulation. However, germination of conidia was only affected at a photoperiod of 24h regardless of the light intensity for both isolates at the two temperatures. No inhibition of germination was observed at 12h photoperiod or full darkness. In IPM systems, *Neozygites* needs to be compatible with chemical pesticides, however, these studies shows that several pesticides affect *N. floridana* negatively.

Contributed Papers Fungi 1 Monday 14:15 **22**

Microbial control of the sweetpotato whitefly with entomopathogenic fungi

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Recently, the Q biotype of tobacco whitefly has been recognized as the most hazardous strain of *Bemisia tabaci* worldwide because of increased resistance to some insecticide groups requiring alternative strategies for its control. We studied the susceptibility of this biotype of *B. tabaci* to 30 isolates of entomopathogneic fungi including *Beauveria bassiana*. These isolates were evaluated on pruned eggplant seedlings, at a concentration of 10^8 conidia/ml. The mortality based on mycosis varied from 18 to 97% after 6 days. An *Isaria fumosorosea* isolate, two *B. bassiana* isolates, and one *L. lecanii* were found the most effective. Furthermore, four isolates were chosen for concentration-mortality response assays and compared to *B. bassiana* GHA as a standard. The numbers of nymphs infected by fungi were correlated with spore concentration. *Lecanicillium lecanii* and *I. fumosorosea* had the shortest LT_{50} at 3.5 and 3.3 days at 6000 ± 586 conidia mm^{-2}. Mortality declined and LT_{50}s were longer as the concentration of conidia was reduced. These results indicated that the Q biotype of sweetpotato whitefly was susceptible to the four isolates of entomopathogenic fungi and these isolates have potential to be developed as microbial pesticides for whitefly control.

Contributed Papers Fungi 1 Monday 14:30 **23 STU**

***Beauveria brongniartii* epizootics on white grubs attacking sugarcane in South Africa**

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Beauveria brongniartii (Saccardo) Petch epizootics were recorded at two sites in the sugarcane producing area of the northern KwaZulu-Natal Midlands of South Africa on the melolonthid species, *Hypopholis sommeri* Burmeister (Coleoptera: Scarabaeidae). To identify the disease-causing fungus, 17 different fluorescently-labelled microsatellite PCR primers were used to target 78 isolates of *Beauveria* spp. DNA. Microsatellite data resolved two distinct clusters of *Beauveria* isolates which represented the *B. bassiana senso stricto.* (Balsamo) Vuillemin and *B. brongniartii* species groups. These groupings were supported by two gene regions, the nuclear ribosomal Internal Transcribed Spacer (ITS) and the nuclear Bloc gene of which 23 exemplar *Beauveria* isolates were represented and sequenced. When microsatellite data was analysed, 26 haplotypes among 58 isolates of *B. brongniartii* were distinguished. Relatively low levels of genetic diversity were detected in *B. brongniartii* and isolates were shown to be closely related. No genetic differentiation was observed between the two sites which represented the Harden Heights and Canema populations. These two sites/populations may be considered one, structured but fragmented population over a distance of 5.5 km's. Historically high levels of gene flow from swarming *H. sommeri* beetles is the proposed mechanism for this observed lack of genetic differentiation between populations. Microsatellite analyses also showed that *B. brongniartii* conidia were being cycled from epigeal to subterranean habitats and *vice versa* in the environment by *H. sommeri* beetles. This is the first record of this species of fungus causing epizootics on melolonthid larvae and adults of *H. sommeri* in South Africa.

Contributed Papers Fungi 1 Monday 14:45 **24 STU**

Potential of entomopathogenic fungi as bed bug control agents

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A series of bioassays were conducted on the human bed bug *Cimex lectularius*, to evaluate the efficacy of *Beauveria bassiana* as a residual biopesticide treatment. An oil formulation of *B.*

bassiana conidia was applied to HP™ Color-Laser Paper or jersey knit cotton at a rate of 3 x 10^6 conidia/cm^2 using an airbrush sprayer. Bed bugs were exposed to sprayed substrates for 1 hr, then transferred to an unsprayed environment and monitored for mortality over a period of 21 days. Bioassays were conducted to evaluate the effect of bed bug strain, sex, life stage, and exposure substrate type on susceptibility to fungal infection and conidial acquisition. The results demonstrated rapid infection and mortality of exposed bed bugs regardless of strain, sex and life stage (mean survival time 4.1-5.6 days) and improved conidial acquisition and more rapid mortality of bed bugs when exposed to treated jersey knit cotton (mean survival time 3.03 days). A further assay revealed the potential for autodissemination of conidia, whereby recently exposed bed bugs transferred spores to unexposed bed bugs within resting harborages, resulting in substantial additional mortality. These results suggest interesting possibilities for use of *B. bassiana* within novel integrated bed bug management strategies.

Contributed Papers Fungi 1 Monday 15:00 **25**

Development of strategies for the incorporation of mycopesticides into the integrated management of *Diaphorina citri* (Hemiptera: Psyllidae)

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The citrus greening disease also known as Huanglongbing is the most devastating problem for the citrus industry in Brazil lately. Control of the vector *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) requires intensive use of chemical pesticides. The need for the incorporation of alternative control methods of *D. citri* has led to a comprehensive effort for development of mycopesticide formulations for this pest. Screening of potential candidates resulted in the selection of a strain of *Isaria fumosorosea* and a strain of *Beauveria bassiana*. Virulence assays with different stages of *D. citri* demonstrated that nymphs were considerably more susceptible to these fungi than adults. Tank-mix adjuvants for aqueous sprays were selected and oil formulations were tested. Efficiency of the fungi was associated with good coverage of aqueous sprays on insects and unsatisfactory results were obtained with oils. Considering the timing and frequency of normal commercial spray practices for pest and disease in citrus, *I. fumosorosea* and *B. bassiana* must be compatible with chemicals pesticides. Laboratory bioassays indicated that several pesticides can be tank mixed with these entomopathogens. Field (n= 3) and semi-fields (n= 2) sprays on adults confined in voile bags on citrus groves using 10^{12} conidia/hectares caused total mortality higher than 72% for both fungi. Following these successful trials, we are currently developing strategies for the incorporation of mycopesticides into IPM in citrus.

Contributed Papers Fungi 1 Monday 15:15 **26**

***Isaria fumosorosea* for control of fruit moths: Comparison of submerged spores and aerial conidia**

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Within a national funded project for biological control of *Cydia funebrana* we investigated the potential of artificial hideouts treated with entompathogenic fungi. After screening various entomopathogenic fungi against a range of fruit moths, *Isaria fumosorosea,* strain Pfr4, gave the best result under laboratory conditions and was selected for further investigations. For development of a production system for Pfr4 we compared liquid and solid state fermentation. In terms of spore yield best results were obtained in liquid culture by using a modified medium described by Samsinakova (1966). Additional results on spore formation will be presented. The production of aerial conidia was not sufficient. When the efficacy of conidia and submerged spores was compared submerged spores were slightly better than aerial conidia. One constrain of the application of living microorganisms is the environmental stability. Therefore, experiments on the persistence of Pfr4 on bark mulch were investigated over two months under semi-field conditions. Despite intensive UV radiation and heavy rainfall a long persistence over the whole experimental time of both aerial conidia and submerged spores was investigated. Further laboratory experiments underlined that the humidity of the mulches is important for the efficacy of Pfr4 and humidity requirements were comparable for submerged spores and conidia. Additional results indicate that water based formulations show an interesting alternative to former preferred oil based formulations. For further comparison of aerial conidia and submerged spores results on viability after drying of and their storability will be presented.

Contributed Papers Fungi 1 Monday 15:30 **27**

Selection of promising fungal biological control agent of the western flower thrips *Frankliniella occidentalis* and development of application strategy

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Larvae of *Frankliniella occidentalis* are known to be refractory to fungal infection. To identify isolate(s) virulent to *F. occidentalis* larvae, *Metarhizium anisopliae* and *Beauveria bassiana* isolates were screened against second-instars of *F. occidentalis*. Conidial production and genetic polymorphism were also investigated. *M. anisopliae* isolates ICIPE 7, 20, 69 and 665 had the shortest LT_{50} values of 8.0-8.9 days while isolates ICIPE 69, 7 and 20 had the lowest LC_{50} values of 1.1×10^7- 3.0×10^7 conidia ml^{-1}. Isolate ICIPE 69 produced significantly more conidia than the others. Alignment of ITS sequences showed differences in nucleotide composition of ICIPE 69 compared to other isolates with absence of a restriction site for Sfo1. An inoculation device baited with or without thrips kairomone (LUREM TR) was tested for delivery of inoculum in the screenhouse. The mean of conidia acquired by single insect was higher ($5.0 \pm 0.6 \times 10^4$ conidia/insect) in the fungus-treated kairomone-baited device than in the device without kairomone ($2.2 \pm 0.4 \times 10^4$ conidia/insect). Thrips mortality was higher in the fungus-treated kairomone-baited device (59.3 ± 3.9%) than in the device without the kairomone (41.7 ± 3.5%). However conidial viability was affected in fungus-treated kairomone-baited device 7 days post-treatment, suggesting an anti-fungal property of the kairomone volatiles. Thrips density per plant was significantly reduced in both treatments with fungus-treated device with and without kairomone as compared to the control. These results demonstrate the prospects of autoinoculation strategy for the control of thrips with *M. anisopliae*, particularly in screenhouses.

Contributed Papers Fungi 1 Monday 15:45 **28**

Comparison of microsclerotia production by various *Metarhizium* species.

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Microsclerotia (MS) production by various species and strains of *Metarhizium* was evaluated by growing cultures in liquid media with 36 g L^{-1} carbon and carbon-to-nitrogen ratios (CN) of 10:1. 30:1, or 50:1. Species evaluated included *M. anisopliae, M. brunneum, M. pingshaense, and M. robertsii*. Biomass accumulation and MS concentrations were measured after 4

and 7 days growth. Microsclerotia-containing biomass was harvested from 7 day-old cultures, mixed with diatomaceous earth (3% w/v), and air-dried to less than 4% moisture. Air-dried MS granules were vacuum packaged and stored at 4 °C for 12 months with viability and conidia production measured over time. All the *Metarhizium* species tested produced MS, although the yield varied by species, isolate, C:N ratio, and fermentation time. Yields of MS ranged from 0.2 - 17 x 10^6 MS L^{-1} after 4 days growth and 0.6 - 62 x 10^6 MS L^{-1} after 7 days growth. Conidia production by air-dried MS granules rehydrated on water agar and incubation at 28 °C for 8 days varied by *Metarhizium* species and isolate, culture medium, and storage age. Highest conidial yields were obtained by *M. pingshaense* cultures (9.0 x 10^9 conidia g^{-1} MS granules after drying and 9.8 x 10^9 conidia g^{-1} MS granules after 12 months' storage). Our studies have shown that numerous *Metarhizium* species produced MS under appropriate culture conditions and that conidia production by MS granules was dependent on the isolate selected and culture conditions employed.

Contributed Papers Monday, 14:00-15:30

Nematodes 1

Contributed Papers Nematodes 1 Monday 14:00 **29**

Mass culturing *Steinernema yirgalemense* using *in vitro* liquid technology

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In Europe the first steps towards an outdoor commercial biopesticide application have been taken using entomopathogenic nematodes. Various nematode formulations against a wide range of insect pests are commercially available. For the quantities needed for field application against pest insects, *in vitro* liquid culturing methods need to be developed. For this study *Steinernema yirgalemense,* an isolate endemic to South Africa, was mass cultured *in vitro.* In order to culture *S. yirgalemense* in liquid eggs was isolated. This was done by infecting *Galleria mellonella* larvae with *S. yirgalemense*. The infected larvae was dissected after 5 days and gravid females was isolated from the dead insects. The associated bacteria of *S. yirgalemense* was characterized, sub-cultured and stored in a viable state. Monoxenic cultures of nematodes and associated bacteria were established on lipid agar plates. The Erlenmeyer flask method was used to evaluate *S. yirgalemense* in the liquid culture. For evaluation of nematode reproduction, a 1 ml sample was taken each day of which 100 µl aliquots were diluted with Ringer's solution and counted. The fundamental life table traits of *S. yirgalemense* and its symbiotic *Xenorhabdus* bacteria specie were defined. The population dynamics and yield of the liquid culture process were assessed. The results obtained during this study will be discussed.

Contributed Papers Nematodes 1 Monday 14:15 **30**

Slug parasitic nematodes for biocontrol of the invasive slug *Arion vulgaris*

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The introduction and subsequent spread of the invasive slug *A. vulgaris* (also known as *A. lusitanicus*) to several countries shows its great capacity for dispersal. Introductions are often accidental being easily transported on various materials (eg. Potted plants). The species has become a major nuisance pest in many countries and causes plant damage in private gardens, horticultural fields and agricultural crops. Another negative impact is the apparent decline of native slug species (eg *Arion ater*). Management of this pestiferous slug is difficult once established. Molluscicides are commonly used as a control measure, as well as cultural techniques, however these measures don't always work. Biological control using the slug parasitic nematode *Phasmarhabditis hermaphrodita* is an additional option. Laboratory experiments have shown that *P. hermaphrodita* is only effective against young stages of the slug (<1 g). Ongoing studies aim to improve the use of *P. hermaphrodita* by adding nematodes to slug baits so as to ensure slug-nematode contact. Initial results appear promising however there are challenges involving the lifecycle of the nematode that need to be addressed.

Contributed Papers Nematodes 1 Monday 14:30 **31**

Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae): Efficacy against *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) in Georgia

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Entomopathogenic nematodes (EPNs) are known as effective biological control agents against a number of insect pests. Efficacy of the EPNs, *Steinernema feltiae* and *Heterorhabditis bacteriophora* obtained from e-nema company (Germany), was evaluated against second instar larvae of the Colorado potato beetle, *Leptinotarsa decemlineata,* and adults of the greenhouse whitefly, *Trialeurodes vaporariorum*, which are key insect pests of potatoes and greenhouse plants, respectively, in Georgia. Experiments were conducted under laboratory and greenhouse conditions for both insect pests, and insect mortalities were checked daily over 3 days. After 3 days*, S. feltiae* provided 85% mortality in the laboratory and 65% mortality in the greenhouse against *L. decemlineata*, whereas *H. bacteriophora* resulted in 60% mortality in the laboratory and 55% in greenhouse. *S. feltiae* gave 70% and 60% mortality in the laboratory and greenhouse, respectively, against *T. vaporariorum*. On the other hand, *H. bacteriophora* provided 50% and 45% mortality in the laboratory and greenhouse, respectively, against the whitefly. *S. feltiae* showed significantly more mortality than *H. bacteriophora* against *L. decemlineata* and *T. vaporariorum* in both laboratory and greenhouse experiments. The results suggest that *S. feltiae* has a better biological control potential against *L. decemlineata* and *T. vaporariorum* than *H. bacteriophora* in Georgia. However, the data also suggest that the nematodes need to be combined with other control agents to be effective against these pests and that further large-scale experiments are needed to determine their practicality under greenhouse and field conditions.

Contributed Papers Nematodes 1 Monday 14:45 **32**

Virulence of entomopathogenic nematodes to larvae of the guava weevil, *Conotrachelus psidii* (Coleoptera: Curculionidae).

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The guava weevil, *Conotrachelus psidii*, is a major pest of guava in Santander-Colombia and causes 100% of losses in fruit quality. This weevil is difficult to control because adults emerge over a long period, and larvae develop to the fourth-instar inside the fruit and move to the soil for pupation. We evaluated the virulence of entomopathogenic nematodes to fourth-instar

larvae by comparing their susceptibility to seven species or strains: *Steinernema websteri* JCL006, *S. colombiense* SNI0198, S*teinernema* sp1. JCL024, S*teinernema* sp2. JCL007, S*teinernema* sp3. JCL027,*Heterorhabditis bacteriophora* HNI0100 , *Heterorhabditis* sp. SL0708. In petri dish assays with sterile sand at a concentration of 200 infective juveniles (IJs) of a given nematode species/strain, larval mortality ranged from 55 to 85%, with *Heterorhabditis* sp. SL0708, S*teinernema* sp3. and *S. colombiense* SNI0198 being the most virulent. In sand column assays with *Heterorhabditis* sp. SL0708 and S*teinernema* sp3. JCL027 at concentrations of 0, 200, and 500 IJs, mortality was greater than the control for *Heterorhabditis* sp. SL0708 (80%) and S*teinernema* sp3. JCL027 (30%) at the lowest concentration. In a greenhouse study with guava trees in 20-L pots, 4 weevil larvae per pot, and concentrations of 0, 1, 2, 4 or 6 cadavers Galleria mellonella of S*teinernema* sp3. JCL027 or *Heterorhabditis* sp. SL0708 caused 40 and 70% mortality at the two highest concentrations. These results show that *Heterorhabditis* sp. SL0708 is virulent to fourth-instar larvae and has potential as a biological control agent in management programs of guava crop in Colombia.

Contributed Papers Nematodes 1 Monday 15:00 **33**

Control of *Conotrachelus psidii* (Coleoptera: Curculionidae) with insect cadavers of *Heterorhabditis* sp1. SL0708 (Nematoda: Rhabditida).

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The guava weevil, *Conotrachelus psidii*, is a major pest of guava in Santader-Colombia causing 100% of losses in fruit quality. We evaluated its susceptibility to *Heterhorhabditis* sp1. SL0708 infective juveniles (IJs) under field conditions applying the nematodes in cadavers of seventh instar *Galleria mellonella* larvae. Field persistence of these nematodes in the soil was evaluated through *G. mellonella*-baiting. Significance differences were observed in the field between control and treatments only when four or six cadavers per 0.25 m2 were applied, caused 90% of mortality. Infective juveniles from the cadavers persisted 12 weeks after application in the field, but decreased greatly thereafter. Our work demonstrates that *Heterhorhabditis* sp1. SL0708 IJs emerging from *G. mellonella* cadavers can be efficacious against guava weevil fourth instar larvae. Also, we demonstrated the long-term persistence of IJs in the soil.

Contributed Papers Nematodes 1 Monday 15:15 **34**

Compatibility between entomopathogenic nematodes and a neem-based product

Elder S. P. Batista, Ana Carolina P. Veiga, Crislany L. Barbosa, Nara E. L. Rodrigues, Ricardo A. Calore and Ricardo A. Polanczyk

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Entomopathogenic nematodes and neem-based products can be used against pests at greenhouses according to the rules of IPM system management. Due to the possibility to use both together and the fact that they have some pests as hosts, it's necessary to evaluate the compatibility between them. The compatibility was evaluated between *Steinernema feltiae* and *Heterorhabditis amazonensis* and a commercial neem-based product (Azamax®). Both nematodes were reared in *Galleria mellonella* larvae. Nematodes infective juveniles were exposed to three neem oil concentrations, according to the recommendation to the chrysanthemum crop (0,3%), the half (0,15%), 2-fold (0,6%), and the control (without neem oil) kept in test-tubes under 25ºC during 48 hours. After this exposure period the nematodes viability and pathogenicity to *G. mellonella* were evaluated. The viability of both nematodes was not statistically different to the control, except to the highest concentration of neem oil, ranging from 74,8% (*S. feltiae* under highest concentration) to 99,4% (*S. feltiae* control group). Both nematodes were pathogenic to *G. mellonella* larvae in the nematodes presence and the mortality was 84% and 88% to *H. amazonensis* assayed with 0.15% and 0.6%, respectively and 100% to the other one. Both nematode species are compatible with neem and can be spread in combination, simultaneous or subsequently to improve the IPM system management.

POSTER SESSION 1 Monday, 16:30 – 18:30

BACTERIA

Poster - Bacteria Monday 16:30 **B-01 STU**

Interaction between Cry1Ia and Vip3Aa proteins from *Bacillus thuringiensis* towards larvae of *Spodoptera* spp. (Lepidoptera)

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Poliphagous pests belonging to the genus *Spodoptera* are considered some of the most important and harmful pests due to its wide distribution over the American continent. Presently with the increase of the usage of genetically modified plants, containing *Bacillus thuringiensis* genes, which code for insecticide proteins, there is the possibility of the surge of resistant insects. To overcome this possibility, it has been proposed to use pyramided plants expressing toxins with different mode of action. Within mind the present study has verified the mode of action of the proteins Cry1IAa and Vip3Aa against neonate larvae of *S. frugiperda, S. albula, S. eridania* and *S. cosmioides* and the interaction between these toxins towards membrane receptors of the intestinal epithelium apical cells. It was then analyzed the biotinilated toxins union to the brush border membrane vesicles (BBMV) of the larvae from these insects. On all of them it was observed using the ligant blotting procedure a putative receptor with approximately 65 kDa in weight. Using in vitro competition assays with the biotinilated toxins, it was possible to observe that the protein Vip3Aa does not compete with the protein Cry1Ia and that the toxin Vip3Aa is more efficient than Cry1Ia when assayed individually. When analyzed together it was possible to observe a synergic action of these proteins over *S. frugiperda*, *S. albula* and *S. cosmioides*. However, for *S. eridania* it was observed competition between these two proteins (Cry1Ia and Vip3A) and a common receptor. Also for this last observation the Cry1IAa protein had a stronger action than the Vip3Aa although with antagonic action when tested together. The results seem to indicate that the use of these genes together, aiming transgenic pyramided plants may not be so effective as need to avoid the surge of resistance by the analyzed pests.

Poster - Bacteria Monday 16:30 **B-02**

Overexpression of the toxin Cry10Aa in a wild-type *Bacillus thuringiensis* svar. *israelensis* strain.

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The mosquitocidal activity of *Bacillus thuringiensis* svar. *israelensis* (Bti) strains is based on a combination of endotoxin proteins (Cry4A, Cry4B, Cry11A, and Cyt1A), located in the

parasporal crystal. However, the type strain contains other endotoxin genes, such as the *cry10A* gene, which is, at the most, slightly expressed. Recently, our group was able to clone and express the Cry10A protein as an inclusion. In this report, the gene construct that made able such an expression (pSTAB-Cry10Aa), was transferred to the wild-type strain of Bti, by electroporation. The presence of this construct was corroborated in five transformant strains (Bti-Cry10-T3, Bti-Cry10-T4, Bti-Cry10-T5, Bti-Cry10-T7, and Bti-Cry10-T10) by the amplification of the Cry10A gene and the erythromycin gene from the vector. Overexpression of the Cry10A protein was deduced by the formation of small inclusions, additional to the usual amorphous crystal, under phase contrast microscopy. Also, SDS-PAGE analysis of spore-crystal complexes showed two bands, corresponding in size to the expected MW from the expression of ORFs 1 and 2 of the Cry10A operon, bands that were absent in the untransformed wild-type strain. Bioassays of spore-crystal complexes from strains Bti-Cry10-T4, Bti-Cry10-T7, Bti-Cry10-T10, and the untransformed wild-type strain, showed estimated LC_{50}s of 5.74, 18.44, 11.28, and 12.0 ng/ml, respectively. Interestingly, strain Bti-Cry10-T4 showed twice the toxicity of the untransformed wild-type strain, and the highest Cry10A expression in the SDS-PAGE analysis. This added toxicity might be due to the overexpression of the Cry10A protein in this strain and/or its synergism with other endotoxins present in the wild-type strain.

Poster - Bacteria Monday 16:30 **B-03**

Effect of Hexanoic acid plant treatment on Cry3Aa toxicity against CPB

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Hexanoic acid is an inducer of plant responses with a potential wide-spectrum of action that acts by means of a priming mechanism. In this work we have studied the effect of the priming activity of Hexanoic acid treatments on Solanaceae plants in the efficacy of *Bacillus thuringiensis* Cry3Aa toxin against Colorado potato beetle (CPB) larvae feeding on Hexanoic acid treated plants. The feeding performance of CPB on three Solanaceae plants (potato, egg-plant and tomato) was assessed. Potato plants showed improved plant quality for this insect as evidenced by larvae weight gain. When larvae were treated with Cry3Aa toxin, higher mortality was found in tomato and egg-plant fed insects compared to potato (88±6%, 77±7% and 59±5%, respectively). Bioassays to test the effect of Cry3Aa on CPB fed on Hexanoic acid treated potato plants were performed on insects adapted or not to feed on Hexanoic acid treated plants. Cry3Aa mortality was 74±2% in adapted larvae and 27±5% and in non adapted larvae, whereas bioassays with insects fed on Hexanoic acid untreated control plants yielded 59±5% mortality. Results indicate that insects adapted to Hexanoic acid primed plants were more sensitive to Cry3Aa toxin. No significant differences were found on CPB feeding performance among the experiments. Furthermore, we analysed the gut juice protein profiles of larvae reared on the three Solanaceae plants induced or not with Hexanoic acid. Differences were observed among all assayed conditions and also on the gut juice proteolytic activity on Cry3Aa toxin.

Poster - Bacteria Monday 16:30 **B-04 STU**

Two cadherin repeat containing proteins are Cry3Ba toxin functional receptors in *T. castaneum*

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Bacillus thuringiensis based bioinsecticides are broadly used due to their utility for environmentally friendly control of insect pests in agriculture. The coleopteran model insect *Tribolium castaneum* is a major global pest of stored products for human consumption for which many genetic and genomics tools have been developed, so it constitutes an ideal subject for the identification of new biopesticide targets based in *B. thuringiensis*. In this work, using *T. castaneum* brush border membrane vesicles (BBMV) we carried out receptor-binding and ligand-blot experiments with the coleopteran specific toxin Cry3Ba, previously shown to be active against this insect pest. Using LC-MS/MS spectrometry, we have identified several BBMV binding proteins as putative Cry3Ba toxin receptors. Among these, we found an aminopeptidase N and a cadherin, already described as receptors for Cry toxins in other insects, and a sodium solute symporter. We analyzed the expression profile of the putative receptor genes and two larval stages were chosen to perform RNA interference experiments with dsRNA. Knockdown efficiency was assessed by quantitative PCR, and dose-mortality assays with Cry3Ba toxin were performed with control and silenced larvae. Results confirmed the role of cadherin and sodium solute symporter as Cry3Ba toxin receptors. Interestingly, the novel Cry toxin receptor sodium solute symporter presented in this work also contains a cadherin repeat domain.

Poster - Bacteria Monday 16:30 **B-05**

Cry3Aa toxin interacts with Colorado potato beetle prohibitin-1, an essential protein for larval viability

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Bacillus thuringiensis Cry toxins mode of action has been described as a step-wise mechanism which comprises toxin interaction with specific membrane receptors triggering toxicity. Recently, several proteomic approaches have suggested that Cry toxin interaction with other midgut proteins might also play important roles in mediating toxicity. Trough ligand blot, cross-linking and mass spectrometry we have identified a CPB protein homologous to insects prohibitin-1 that binds Cry3Aa toxin. By PCR, using degenerate primers, and RACE we amplified CPB *prohibitin-1* coding sequence, which was compared with other *prohibitin-1* sequences present in databases. To ascertain the role of prohibitin-1 during Cry3Aa mediated intoxication we carried out *in vivo* RNA interference assays. Feeding CPB larvae with dsRNA targeting *prohibitin-1* caused significant larvae mortality and reduced body weight gain. Furthermore, Cry3Aa treatment to *prohibitin-1* dsRNA fed larvae produced a marked increased in larvae mortality. According to these data, quantitative real-time PCR analysis showed that *prohibitin-1* mRNA levels in the midgut tissue were significantly reduced after feeding CPB larvae with specific dsRNA. Taken together, these results suggest that prohibitin-1 protein plays a key role in CPB larvae viability, so that Cry3Aa toxin interaction with prohibitin-1 could interfere with this function, enhancing Cry3Aa mediated intoxication in CPB larvae.

Poster - Bacteria Monday 16:30 **B-06 STU**

***Spodoptera exigua* lectins: a protein family involved in the immune response to different pathogens**

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Unlike vertebrates, the insect defense against pathogens relies mainly in their innate immunity. Therefore, certain lectins, due to their peptidoglycan binding capacities, play a major role in no self recognition as pattern recognition receptors. Transcriptome of the beet armyworm *Spodoptera exigua* larvae revealed the existence of at least thirty- two unigenes containing lectin domains. In order to investigate possible role of the lectins in the immune response, we have chosen twelve of these lectin-related proteins to characterize their sequences, tissue distribution and response to pathogen patterns. Based on

sequence homology, we divided the studied lectins into two groups. The first group includes six lectins with high homology to the other lepidopteran lectins and mainly expressed in the fat body of the larvae. The other six are mostly expressed in hemocytes and share homology with hymenopteran lectins. For a better comprehension of the role of these proteins in the immune system of *Spodoptera exigua*, we studied the expression of these genes in the midgut, fat body and hemocytes of larvae treated with different microbial pathogens or their virulence factors. Results have revealed a specific pattern of regulation according to the pathogen. We have observed some of these lipopolysaccharide binding proteins to be regulated by Vip toxin from *Bacillus thuringiensis*, baculovirus, Gram negative and Gram positive bacteria suggesting that these proteins may play different roles in the immune response of this and related insect species.

Poster - Bacteria Monday 16:30 **B-07**

Degenerate PCR based search for *cry* genes and characterization of novel *cry* genes from *Bacillus thuringiensis*

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We have detected novel genes belong to the *cry* family that encodes insecticidal proteins, by degenerate PCR from known *Bacillus thuringiensis* (Bt) type strains *galleriae*, *tolworthi* and *japonensis*; all of which perform insecticidal activities. Bt produces a parasporal crystal consisting of Cry proteins that are toxic to insects. The toxins of Bt have been used as biological insecticides and are effective tools for the control of a wide variety of pests. Therefore new Bt and Cry proteins with toxic potential against pests are highly sought after around the world. We designed degenerate primers and searched for *cry* genes using the primers. Cry proteins contain eight highly conserved block regions, and degenerate primers were designed targeting specific block regions. Amplified DNA fragments obtained using the primers were of predicted size. The analyses of nucleotide sequences and the deduced amino acid sequences revealed that the obtained sequences were similar to those of *cry* family. Homology searches in the DDBJ database suggest the possibility that the newly-obtained sequences may in fact be novel *cry*. Two candidate fragments from the detected nucleotide sequences from *japonensis* type strain that of two fragments highly potential of novel *cry* were further analyzed to determine the ORF. As a result, deduced amino acid sequences from the genes involved block regions that are characteristic of Cry proteins. Each gene was confirmed to be a novel *cry* family. These results support that degenerate PCR is effective in searching and screening for novel *cry* family of soil bacteria.

Poster - Bacteria Monday 16:30 **B-08 STU**

Endophytic colonization by Brazilian strains of *Bacillus thuringiensis* on cabbage seedlings to control *Plutella xylostella*

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Plutella xylostella (L.) (Lepidoptera: Plutellidae) is a pest of great economic importance, due to losses caused in brassica crops worldwide. The control of this pest is hindered by the selection of insects resistant to various chemicals and by its cryptic habit. The aim of this work was to select new *B. thuringiensis* strains with potential to control this insect, in a systemic way, a new control strategy. The Brazilian *B. thuringiensis* strains were characterized and their ability to colonize cabbage seedlings was verified in different parts of cabbage seedlings. The strains showed high toxicity to *P. xylostella* but no significant differences were observed in the LC_{50} values of S1905, S2122 and S2124 strains when compared to Btk HD-1. All the strains colonized the cabbage seedlings and their spores and crystals were observed on roots, shoots and leaves of cabbage but predominantly on roots. The colonization was shown on the surface, near the stomata and inside stomata pores and was confirmed by tests *with B. thuringiensis* labeled with ^{35}S methionine. The mechanism of penetration of *B. thuringiensis* probably occurs through openings and injuries in roots and followed by movement through the xylem until reaching the leaves. The endophytic colonization of *B. thuringiensis* strains did not affect the germination of seeds and initial seedling development. This article demonstrates for the first time the ability of *B. thuringiensis* strains to colonize cabbage seedlings and its importance in the management of *P. xylostella* through a systemic bioinsecticide.

Poster - Bacteria Monday 16:30 **B-09 STU**

Putative loop 1 in domain II of *Bacillus thuringiensis* Cry39Aa toxin are important for larvicidal activity against *Anopheles stephensi.*

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Bacillus thuringiensis is a rod shaped, gram positive, spore forming bacterium that produces parasporal crystal proteins during sporulation. Cry39Aa from *B. thuringiensis* subsp. *aizawai* BUN1-14 is highly toxic to the mosquito larvae of *Anopheles stephensi*, which transmit malarial parasites. We developed a homology model of the Cry39Aa toxin using known structure of Cry4Ba toxin (PDB file 1W99) as template and identified predicted domain II loop 1 region, ^{349}KYAYWR^{354}. Many studies of different Cry toxins have shown that surface-exposed loop regions of domain II are critically involved in toxicity and receptor recognition. To investigate functional role of loop 1 region of Cry39Aa toxin, we performed site-directed mutagenesis in this region. In larvicidal activity, alanine substitutions revealed that the whole structure of loop 1 region, especially two aromatic amino acids Y350 and Y352, is essential. However, Cry39Aa mutants with phenylalanine substitutions of two tyrosine residues (Y350, Y352) in loop 1 had no significant effect on toxicity as compared to wild type Cry39Aa. Competition binding assay revealed that mutant toxins substituted to alanine showed no significant competition with wild type Cry39Aa toxin binding to *A. stephensi* brush border membrane vesicles. These results suggest that the molecular structure of loop 1 region in domain II of Cry39Aa toxin is important for toxicity and receptor binding to *Anopheles* larval midgut.

Poster - Bacteria Monday 16:30 **B-10**

The adaption evolution and distribution analysis of *cry1I* gene in *Bt* strain

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The *cry* gene was the key factor for *Bacillus thuringiensis* (Bt) infect an insect. And *cry* gene can spread while Bt reproduced in an insect cadaver. While *cry1I* group genes are described as silence genes in Bt. In this report, we analysis the evolutionary characteristics and distribution of *cry1I* group genes in Bt strains, also investigated the relationship between the *cry1I* group genes toxic activity and their distribution.

Poster - Bacteria Monday 16:30 **B-11**

Effects of Vip3Aa and Cry1Ac on enzyme activities in larvae of cotton bollworm *Helicoverpa armigera*

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In order to clarify the action mode of Vip3Aa and provide the theoretical bases for applying Vip3Aa as an important protein in "New toxin strategy", the effects of Vip3Aa and Cry1Ac on protease, detoxification enzymes and aminopeptidase N (APN) activities in larvae of cotton bollworm *Helicoverpa armigera* (Hübner) were compared. The result of bioassay in laboratory showed that the lethal efficacy of Vip3Aa was lower than Cry1Ac, but Vip3Aa had obviously inhibition effect on larval development. The activities of total protease and tryptase were quickly increased after *H. armigera* fed on artificial diet containing Cry1Ac or Vip3Aa or Cry1Ac+Vip3Aa respectively. But compared with control group, these two enzyme activities were not significantly different 12h after treated by Cry1Ac. While the increased activities of these two enzymes in larvae treated by Vip3Aa were clearly prolonged, and the activity of chymotrypsin-like enzyme in this treatment was also higher than control. It indicated that degradation speed of Cry1Ac might be faster than Vip3Aa, and the enzyme systems involved degradation maybe were different. Meanwhile, Cry1Ac and Vip3Aa using together could extend the degradation time. The activities of glutathione-S-transferase and α-naphthalene acetate esterase increased after *H. armigera* fed on artificial diet containing Cry1Ac or Vip3Aa or Cry1Ac+Vip3Aa respectively. It suggested that these enzymes maybe involve the detoxification process of Vip3Aa and Cry1Ac. However, the Vip3Aa and Cry1Ac had little effect on activity of aminopeptidase N, maybe their toxicity action process had no relationship with enzyme activity change of APN.

Poster - Bacteria Monday 16:30 **B-12**

Identification of *cry* genes from *Bacillus thuringiensis* isolates with potential for control of *Ecdytolopha aurantiana* (Lima, 1927) (Lepidoptera: Tortricidae)

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Brazil accounts for 85% of world production of orange juice and 56% of the production of fresh orange. The orange crop is host to many pests, including the *orange* fruit borer, *Ecdytolopha aurantiana* (Lima, 1927) (Lepidoptera: Tortricidae). This insect is present throughout South America and its occurrence is reported in nine Brazilian states. Currently, control of *E. aurantiana* has been performed with chemical, physiological and biological products, including *Bacillus thuringiensis* (Bt) based formulations. The insecticidal activity of Bt-based products is attributed mainly to parasporal crystals which are composed by one or more types of toxic proteins denominated Cry, that show specific toxic activity against insects of various orders. The objective of this study was to identify strains of *B. thuringiensis* with toxic activity against *E. aurantiana*, as well as identify their cry genes. A total of twenty *B. thuringiensis* strains were evaluated by bioassays with first instar larvae of *E. aurantiana*. *B. thuringiensis* var. *kurstarki* HD1 was used as positive control of the assays. Two *B. thuringiensis* isolates (BR09 and BR37) showed more toxic activity than the standard strain HD-1. The cry genes of the strains were characterized by PCR and sequencing. The obtained sequences were identified as cry1 and cry2 genes. Genes belonging to other groups of cry genes are being identified by sequencing aiming to identify genes with toxic activity with potential for control of *E. aurantiana*.

Grants: CNPq and CAPES, Brazil

Poster - Bacteria Monday 16:30 **B-13**

Characterization of *Bacillus thuringiensis* isolates with toxic activity against economically important insect pests in Brazil

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The aim of this study was to characterize *Bacillus thuringiensis* strains that exhibit highly toxic activity against important insect pests in Brazil. The strains were characterized for the morphology of crystals by optic and electron microscopic analysis, the presence of *cry1*, *cry2* and *cry3* genes by PCR and sequencing and the Cry proteins of the crystals by SDS-PAGE analysis. Different strains showed crystals of several forms as bypiramidal, square, round and enclosed crystals. The characterization by PCR allowed the identification of genes belonging to the three groups analyzed. Analysis of the proteins of the spore-crystal mixtures showed the presence of polypeptides of different sizes, ranging from 25 to 130 kDa, and the 130 and 65 kDa were the most commonly found. The characterization of the genes within each class (*cry1*, *cry2* and *cry3*) was made by sequencing. A total of eight different *cry1* genes were identified. The gene *cry1Aa* was the most frequent, being found in 13 of 20 tested strains. The genes *cry2Aa* and *cry2Ab* were detected in eight and two strains, respectively, while the *cry3* genes were found in eight strains, with the possible identification of the gene *cry3Aa* in only two strains. Some strains did not give any PCR product for the analyzed genes and primers for other groups of *cry* genes will be used in order to identify strains that may harbor potentially novel Cry proteins as well as strains with combinations of less frequently observed *cry* genes. (Grants: CNPq and CAPES, Brazil).

Poster - Bacteria Monday 16:30 **B-14 STU**

Relationship between crystal shape and fingerprinting (rep-PCR) of the *Bacillus thuringiensis*

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Bacillus thuringiensis (Bt) is considered the main entomopathogenic bacterium that produces parasporal crystals constituted of insecticidal proteins. Bt is widely used in biological control of pests, as bioinsecticides and in transgenic plants resistant to insect pests. The aim of this work was to analyze 63 Bt strains genetic diversity by rep-PCR, to analyze their crystal shapes and to find some relationship between them. The crystal shapes of all bacteria were observed with a phase contrast microscope. For this study, BOX-PCR, ERIC1/ERIC2-PCR and REP1/REP2-PCR were used. All PCR and thermocycler conditions were conducted as previous papers and the PCR patterns were obtained by electrophoresis in 2% agarose gels. All fingerprinting profiles were generated from at least two independent experiments to determine their reproducibility. A dendrogram was constructed using the Jaccard coefficient and UPGMA with Genes software. After cluster analysis, the cutoff point (43%) was defined and almost all the strains were defined in four distinct groups. All bipiramidal crystal shapes fell into the group 1. The group 2 was composed by only two strains that showed spherical crystal shape. All strains that showed spherical and cuboid crystals were grouped in the group 3, the largest group. The group 4 was composed by strains that showed spherical crystal shapes. Some strains did not group with any other. The analyzed data shows a relationship between the similarity of strains of *B. thuringiensis* and their crystal shapes

Poster - Bacteria Monday 16:30 **B-15 STU**

Detection genes *cry 2* and *cry 9* in strains of *Bacillus thuringiensis* for the control of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae)

Rosane Bezerra da Silva[1], Arthur Augusto Gonçalves Torres[2], Thais Barros Rodrigues[1], André Henrique Campelo Mourão[2], Camila da Silva Fernandes[2], Kátia Gisele Brasil Boregas[3], Fernando Hercos Valicente[3]

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The crystalline inclusions of *Bacillus thuringiensis* along with the spores have a high potential to control a great number of insect pests belonging to different orders. The electrophoretic profile of the PCR products can be used to predict the insecticidal activity of these genes. The aim of this study was to characterize a total of 95 Bt strains, ten were kindly provided by the USDA (United States Department of Agriculture), nine kindly provided by the Institute Pasteur, eight belong to Embrapa Maize and Sorghum Bt bank. Also, 68 strains with no subspecies information (Embrapa Bt Bank) with *Spodoptera frugiperda* mortality above 75% were selected from 4459 isolates. Molecular characterization was PCR based using specific *cry2* and *cry9* primers. Thirty-two percent of the isolates showed amplification of at least one class of *cry2* gene, and 17% for *cry9* gene, and most frequent genes (25%) were *cry2Ab*, *cry2Aa* (14%), *cry9* (13%) and *cry9Aa* (8%), respectively, and the least frequent genes were *cry2Ac* (3%), *cry2Ad* (3%), *cry9A* (3%) and *cry9B* (4%). Three isolates showed higher number of amplification products (HD29 *Bt galleriare,* 1658 and 1657). Our results did not show a relationship between mortality and the presence of *cry* genes, since the isolate HD29 showed low mortality of *S. frugiperda* larvae (12,8%). Our results show the importance of molecular characterization even when isolates show low mortality in the bioassay. These isolates may show genes of agronomic importance and can still be used in future studies, such as genetic transformation of plants.

Poster - Bacteria Wednesday 16:45 **B-16**

Molecular characterization and production of *Bacillus thuringiensis* based biopesticide

André Henrique Campelo Mourão[1], Rosane Bezerra da Silva[2], Camila da Silva Fernandes[1], Thais Barros Rodrigues[2], Arthur Augusto Gonçalves Torres[1]; Kátia Gisele Brasil Boregas[3]; Fernando Hercos Valicente[3]

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Corn is one of the most important agricultural products in Brazil. Pests such as *Spodoptera frugiperda* and *Helicoverpa zea* can be controlled with the use of *Bacillus thuringiensis* (Bt) based biopesticides. This work aimed the growth of *B. thuringiensis* subspecies *tolworhti* and the optimizing of the fermentation process for biopesticide. A carbon/nitrogen ratio 5:1 was used and the media was enriched with mineral salts ($MgSO_4$, $FeSO_4$, $MnSO_4$ and $ZnSO_4$). The pH was adjusted to 7.5, and $CaCO_3$ (calcium carbonate) was added only once to stabilize the pH during fermentation. All the fermentation process was carried out in a fermentator model TEC-BIO 4.5 liters. Samples were collected ranging from 2 to 72 hours after inoculation. Although $CaCO_3$ was added, pH dropped to 5.5 after 8 hours de incubation, optical density reached 1.5 at 600 nm after 48 hours, and cell mass was 1.391 g/L after 48 hours. Within 24 hours of fermentation 8.13 x 10^8 spores / ml were observed. Two-day-old larvae were tested and the results showed that mortality rate was 43.62% for *S. frugiperda* and 82.61% for the *H. zea* larvae. Despite showing more spores/mL 24 hours after inoculation, the highest percentage of mortality was 56 hours after inoculation for both species tested. PCR was performed with this Bt strain that showed *cry1Ab/1Ac,cry 1Aa/1Ad, cry 1B, cry 1D, cry 1Ea/1Eb, cry 1A5, cry 1Ab, cry 1I e cry 2Aa* genes.

Poster - Bacteria Wednesday 16:45 **B-17 STU**

Isolation, diversity, cloning and molecular characterization of *cry* gene contents from *Bacillus thuringiensis* isolates

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Insect infestation causes tremendous loss in the yields of several crops. Chemical pesticides have long-term detrimental effects, leading to environmental degradation and elimination of natural parasitoids and predators. Also, several hundred insect and mite species have developed resistance to one or more chemical insecticides. There is, therefore, a need for environmentally safe pest control to maintain sustainability of the environment. *Bacillus thuringiensis* is the most widely used bacterial bio-insecticide is most promising one in this direction. Insecticidal crystal proteins are useful agricultural tools. Applications towards non-lepidopteran insects are not as common as applications towards lepidopteran insects. In the present study four approaches were employed viz., analysis of crystal protein production with phase contrast microscopy/transmission electron microscopy, detection of *cry* gene content by PCR, SDS-PAGE profiling, Cloning, sequencing and phylogenetic analysis. 200 soil samples were used for isolation of *Bt* and a total of 69 putative isolates of *Bt* that produce parasporal crystalline inclusions were isolated from 5267 *Bacillus* like colonies by Bipyramidal inclusion was predominantly present in 32.2% of the *Bt* isolates when compared to other shapes. Majority of the isolates showed the presence of 50-60 kDa protein bands on SDS-PAGE while the rest showed 130, 73, 34 25 and 13 kDa bands. PCR analysis revealed predominance of Coleopteran active *cry* genes in these isolates. The variations in the nucleotide sequences, crystal morphology and mass of crystal protein(s) purified from the isolates of *Bt* revealed genetic and molecular diversity. Three strains containing Coleopteran active *cry* genes showed higher toxicity against first instar larvae of *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae).

POSTER SESSION 1 Monday, 16:30 – 18:30

DISEASES OF BENEFICIAL INVERTEBRATES

Poster - DBI Monday 16:30 **DBI-01**

Parasitic castration of a marine snail by larval trematodes

Pilar Alda and Sergio R. Martorelli

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In a two-year survey, we collected and dissected 7,504 specimens of *Heleobia australis* (Mollusca: Cochliopidae) from Bahía Blanca estuary, Argentina. We fixed some infected specimens in Bouin's fluid and used standard histological techniques to locate the site of infection of larval trematodes within the snail. The remaining individuals were necropsied to sex the host (by the presence of penis in males), classify females in mature or immature (by the presence or absence of oocytes), and examine the presence of parasites. We found 40% of the snails infected with 15 species of trematodes: Microphallidae (4), Echinostomatidae (2), Lepocreadioidea (1), Heterophyidae (1), Cryptogonimidae (1), Psilostomidae (1), Sanguinicolidae (1), Notocotylidae (1), Haploporidae (1), Renicolidae (1), and other (1). *Microphallus simillimus*, a microphallid with an abbreviated life cycle, was the most prevalent trematode (32%). Except for the sporocysts of *Renicola* sp. that growth in the mantle tissues

and a metacercaria of an undetermined family that encyst in the head of snails, we found that all the parasites were occupying the digestive gland and the gonad of snails. Females were more abundant and infected than males and infected mature females were fewer than uninfected mature females. We suggest that parasites could be affecting the development of the penis in males or causing higher mortalities in males than in females. Our results allow us to support the idea that larval trematodes consume the host energy for their own reproduction, blocking host reproduction.

Poster - DBI Monday 16:30 **DBI-02 STU**

Immunity related genes in honey bees in response to synthetic acaricidal treatments

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The mite Varroa destructor is an ectoparasite affecting honey bees worldwide, causing serious economic damage on beekeeping. Synthetic acaricides have been among the principal tools available to beekeepers for varroa management. Several studies have been shown its negative effects on honey bee physiology even at sub-lethal levels. Recent research suggests that those molecules strongly impact on immune signaling cascades and cellular immunity. However, toxicity studies at immune gene expression levels are few. In the present work, LC_{50} in six-day-old bees were determined for the following acaricides: tau-fluvalinate, flumethrin, amitraz and coumaphos. According to this obtained value, a group of individuals were treated and then processed for qt-PCR analysis. Transcript levels for the genes encoding the antimicrobial proteins hymenoptaecin and defensin, the immunity related proteins phenoloxidase and vitellogenin were assessed. Flumethrin significantly elevated the expression of hymenoptaecin and phenoloxidase. With respect defensin expression, differences became significant when coumaphos vs. flumethrin treated bees were compared, although no differences were detected when comparison was made with control bees. No significant changes were recorded in the expression levels of vitellogenin among bees treated with acaricides and control bees. This work constitutes the first report, under laboratory conditions, about induction of immune related genes in response to synthetic miticides. We discuss possible underlying mechanisms for these results and host susceptibility to different pathogens after acaricide exposure, since some of these molecules frequently have been found in apicultural matrices at high concentrations.

Poster - DBI Monday 16:30 **DBI-03**

The fatal relationship between *Varroa destructor*, DWV, and honey bees

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Deformed wing virus (DWV) is a serious threat to honey bee health if transmitted to pupae by the ectoparasitic mite *Varroa destructor*. Normally, DWV causes covert infections in bees. Overt infections may occur after transmission of the virus to pupae by parasitizing mites. They are characterized by infection of the brain and/or by malformed wings in emerging bees or by pupal death. It has also been convincingly demonstrated that the induction of an overt infection through mite-transmitted DWV is related to DWV replication in the mite prior to transmission. We therefore hypothesized that mites harboring replicating virus, pose a more serious threat to honey bee pupae than mites carrying no or non-replicating DWV. To test this hypothesis, we performed removal assays with hygienic bees and (i) pupal cells infested with highly virulent mites (harboring replicating DWV), (ii) pupal cells infested with less virulent mites (harboring no or non-replicating DWV), and (iii) non-infested pupal cells as control. Pupae parasitized by highly virulent mites were removed at significantly higher rates (54.33±13.91%) than pupae infested by less virulent mites (9.67±5.67%) and control pupae (2.0±1.63%) (Scheffé test, $P<0.001$ in both comparisons). There was no significant difference in the removal rates between the last two categories (Scheffé test, $P>0.1$). These results indicate (i) that mites harboring replicating virus are obviously more virulent than mites harboring no or non-replicating DWV and (ii) that the hygienic behavior of the bees is directed towards the damage done to the pupae rather than towards the presence of the mite.

Poster - DBI Monday 16:30 **DBI-04**

Changes and similarities in the expression of honey bee immune response genes during the infection with two different genotypes of the bee pathogen *Paenibacillus larvae*

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American Foulbrood is caused by the gram-positive spore forming bacterium *Paenibacillus larvae*. This highly contagious and fatal pathogen affects the brood of honeybees and hence has severe impact on the whole colony. Infections are triggered by the ingestion of spore contaminated food. These spores germinate and massively proliferate in the midgut until a critical point is reached at which the bacteria breach through the epithelium into the haemocoel, kill the larva, and decompose the remaining cadaver to a ropy mass. There are four different genotypes (ERIC I-IV), but only ERIC I and ERIC II are prevalent. Phenotypical differences of these genotypes are detected in metabolism, colony morphology and virulence. Furthermore, there is strong evidence that ERIC I and ERIC II use different mechanisms during the pursuit of infection and the actual killing of the host. It is of immense interest to investigate if these differences between the *P. larvae* genotypes are reflected in the immune reaction of the honey bee larvae during infection. Research conducted so far utterly neglected these different genotypes. Dissecting the differences or similarities could not only help to understand the course of infection with *P. larvae*, but might even add to the knowledge of the immune response in honey bees as such. Results comparing transcription levels of several immune genes will be shown.

Poster - DBI Monday 16:30 **DBI-05**

Infectious agents of *Litopenaeus vannamei* (Boone, 1931) and their relationship with physicochemical parameters in three different culture systems in Gulf of Mexico, Mexico

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The present study assessed the prevalence of infectious agents in *Litopenaeus vannamei* culture systems (intensive, semi-intensive, and extensive) on farms located in Tamaulipas, Mexico. We also analyzed the statistical relationship between

these agents and the physicochemical parameters related to the aquatic environment during the summer-autumn of 2008 and the spring–summer of 2009. *Acineta* sp. at the Reynosa farm, showed a significant correlation with temperature and was the only protozoan found at this farm, which uses semi-intensive culturing with underground water, and showed the best conditions related to lower impacts of infectious diseases. La Pesca shrimp farm uses water from the Laguna Madre estuary and an extensive culture system. *Epistylis* sp. at the La Pesca farm and the Morón farm showed a significant relationship with turbidity; the dependence of *Nematopsis* sp. on temperature at La Pesca was highly significant. The filamentous bacterium, *Leucothrix mucor*, was found at the Morón farm, which uses an intensive production system with fresh water from the Tigre River. To our knowledge, this report is the first to analyze the relationship of this filamentous bacterium with water quality. These results could provide important information for future epidemiological studies to facilitate better resource management and the prevention of infectious or parasitic diseases in farmed shrimp.

Poster - DBI Monday 16:30 **DBI-06**

***Apis mellifera* cellular immune responses in-vitro: Qualitative differences before and after the pupae metamorphosis black box**

Pedro Negri[1,3], Matias Daniel Maggi[1,3], Natalia Fernandez[1,3], Lorenzo Lamattina[2,3] and Martin Javier Eguaras[1,3]

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Insects have a well developed innate immune system to defend themselves from infectious organisms. Innate immune responses are subdivided into humoral and cellular defences. Most literature is focused on model species like *Drosophyla melanogaster*, selected Lepidoptera and mosquitoes. The honey bee *Apis mellifera* (Hymenoptera) is an ideal system for investigating ontogenetic changes in the immune system. It combines holometabolous development within a eusocial caste organization. Most work to date has focussed on the humoral immune response of *A. mellifera* while very little is known about the cellular immune response of honeybees. The main purpose of this work was to study *A. mellifera* hemocytic responses before and after the pupae metamorphosis. Haemolymph from the *A. mellifera* Sppining larvae (SL) and newly emerged workers (W) was used to perform the experiments. Cells that spread upon stimulation with Lipopolysacharide, flagellin, *Paenibacillus larvae* and contact with a glass surface were considered as immune related hemocytes. Hemocytes were observed using DIC microscopy and time lapse imaging and described in concordance to Strand 2008. Noticeable differences were found between SL and W hemocytes in regard to appearance, size, cell migration and spreading in-vitro. However, SL and W hemocytes described similar encapsulation related behavioural pattern. Previous reports showed quantitative differences between *A. mellifera* SL and W hemocytes. This work shows qualitative differences and similarities between SP and W. Nevertheless, in-vitro *P. larvae* phagocytosis is reported in this work. Also, an experimental system for performing in-vitro encapsulation experiments with *A. mellifera* hemocytes in particular is proposed here.

Poster - DBI Monday 16:30 **DBI-07**

Survey for *Nosema* spp. in Belize Apiaries

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Nosema ceranae is a recently described microsporidian pathogen of honey bees that has been implicated in honey bee losses in the United States and Europe. To date, only one survey in a Central American country (i.e. Costa Rica) has been conducted on the incidence of *Nosema* in honey bees. Due to the widespread prevalence of *Nosema ceranae*, we sought to determine whether *N. ceranae* was present in Belize and if so, how prevalent and at what levels. In the spring of 2010, four apiaries in two adjacent districts of Belize were assessed with ten colonies sampled from each apiary. Bees were analyzed for *Nosema* spp. by spore counting and real-time PCR for species determination. Overall, we found very low levels of *N. ceranae* with an average of 0.87 ± 0.68 *N. ceranae* DNA copies across all samples, and 5.77 ± 1.72 when only analyzing positive samples. *Nosema apis* was not detected in any sample. Samples from apiaries 1–4 had 45%, 5%, 0%, and 10% positive samples, respectively. Our findings in Belize are different from other studies as *N. ceranae* levels were not only low, but infections were not prevalent (average of 15% of colonies infected). This difference could be due to the time of year sampled. Our findings seem to indicate that honey bees in Belize are relatively free of *Nosema* and thus, appear to be healthier than honey bees from adjacent Central American countries. Further studies on the incidence of *Nosema* are recommended in other neighboring countries.

Poster - DBI Monday 16:30 **DBI-08 STU**

Evaluation of the toxicity of essential oil components on *Varroa destructor* (Acari: Varroidae) and *Apis mellifera* (Hymenoptera: Apidae)

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The parasitic mite *Varroa destructor* is considered to be one of the most serious pests of the honey bee *Apis mellifera*, causing great economic losses to the beekeeping industry. Historically, synthetic acaricides such as pyrethroids and organophosphates have been the miticides of choice for mite control in apiaries. Nevertheless its widespread use, and often its misuse throughout those years, has placed a strong selective pressure on mite populations, and therefore, resistant populations have emerged in several countries worldwide. Alternative acaricides offer a highly desirable alternative to the synthetic products. In adition, information about toxicity of essential oil components against *A. mellifera* and *V. destructor* is still scarce. The lethal effects (LC_{50}) of mircene, carvacrol, phellandrene, cinnamic aldehyde, eucalyptol and thymol were assessed in this work. The acaricidal effects (LC_{50}) of thymol and phellandrene against *V. destructor* were tested also. These components were selected due they are the major components of essential oils that showed promisors acaricidal effect on *Varroa* mites in previous researches. Bioassays were performed to assess oil components toxicity by total exposure over bees and mites at 24, 48 and 72 h. Results showed that essential oil components differ in their bioactivity against the target arthropod. Thymol and carvacrol was the most toxic components for *A. mellifera* while eucalyptol and phellandrene were the less toxic for bees. With regard mite toxicity thymol was the most toxic component for the parasite. Oil components bioactivity against mites demonstrates that these substances represent a great alternative for future formulations for *V. destructor* control.

POSTER SESSION 1 Monday, 16:30 – 18:30

FUNGI

Poster - Fungi Monday 16:30 **F-01**

In vitro* activity of *Laurus nobilis*, *Calamintha officinalis* and *Lippia alba* against *Ascosphaera apis.* Evaluation of the potential toxic effects on adults and larvae of *Apis mellifera

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Chalkbrood is a disease of the honeybee (*Apis mellifera* L) caused by the fungus *Ascosphaera apis.* The activity of the essences of *Laurus nobilis*, *Calamintha officinalis* and *Lippia alba* was evaluated *in vitro-in vivo.* The potential toxic effects of the essences on adults and larvae of *A. mellifera* L were also studied. The *in vitro* activity of different concentrations of essences (50, 100, 200, 400, 800, 1600 ppm) was tested against seven isolates of *A. apis*. The Lethal dose 50 (LD50) of essences on adult bees was calculated. The doses employed were: 0.25; 0.50; 1; 2; 4; 8 µg a.i/bee. Dimethoate and sucrose in water solution were used as a toxic and the non-toxic control. Mortality was assessed after 24, 48, and 72 h. The LD50 was calculated by PROBIT. To evaluate the toxicity on larvae, an area of 180 brood cells (instars 1–5) was marked, at day 0, in each colony and larval mortality was assessed at 7 and 21 days after the application of treatments by counting number of eggs, open brood, sealed brood, and empty cells. ANOVA and LSD tests were used. The susceptibility profile of isolates studied not showed significant differences. Thus, after 72h the fungal growth was inhibited by *L. nobilis, C. officinalis* and *L. alba* at 1600, 800 and 800 ppm respectively. The LD50 of the essences on adult bees showed "virtually no-toxicity". The *in vivo* essay of larval toxicity showed that *C. officinalis* was the only essence no toxic to larvae.

Poster - Fungi Monday 16:30 **F-02**

Effect of *in vitro* successive subcultures of *Beauveria bassiana* to U.V. tolerance

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The aim of this study was to assess the effect of successive subculture, *in vitro,* of two isolates of *Beauveria bassiana* (CG 152 and CG 40 Unioeste), in different nutritional conditions on UV radiation tolerance. PDA (potato dextrose agar) and MPE (medium to produce *Beauveria* spp. conidia) media were used. The fungus was initially inoculated in *Alphitobius diaperinus* adults (1st(A)), subcultured for 20 times in the different media, inoculated in the insect for the second time, and subcultivated again in the different media (1st(B)). Conidia from the 1st (A), 10th, 20th and 1st (B) subcultures of both isolates were selected for tests. Petri dishes containing MPE medium were inoculated with a suspension (1×10^3 conidia ml^{-1}) and exposed to UV radiation for 0 and 1 minute. After four days the number of colony forming units was assessed. The *B. bassiana* sensibility to UV radiation was influenced by successive subcultures. The isolate CG 152 was more tolerant to UV radiation when cultivated in MPE. However, when cultivated in PDA medium, the tolerance was reduced after the 10th subculture, and there wasn't a recovery of tolerance to UV after inoculation in host. The isolate Unioeste 40 cultivated in PDA medium wasn't affected after successive subculturing. When cultivated in MPE medium, there was an increase in UV radiation tolerance after the second host inoculation 1st (B). Therefore, we observed that the influence of the successive subculturing in the radiation tolerance depends on the isolate used and the culture medium.

Poster - Fungi Monday 16:30 **F-03**

Influence of the nutritional conditions on *Beauveria bassiana* (Bals.) Vuill. tolerance to temperature

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The efficiency of entomopathogenic fungi in biological control depends mainly on environmental conditions and the highest temperatures are one of the most limiting factors. The aim of this study was to develop culture media for *B. bassiana* conidia production and correlate its nutritional aspects with the tolerance to high temperature. The media was made of *Alphitobius diaperinus* adult insects (MAD 10%) and a nutritional solution used in the hydroponic cultivation of plants (SNS 25% and SNH 25%). PDA (potato dextrose agar) and MPE (medium to produce *Beauveria* spp. conidia) were also used. The conidia from isolate Unioeste 4, produced in different media remained in a climatized chamber at 30 ± 1 ° C for 0, 5, 10, 15 and 20 days. A suspension (1×10^7 conidia ml^{-1}) was applied on Petri dishes containing PDA medium and the conidia viability was assessed after 20 h quantifying the germination. The temperature tolerance was positively correlated with K, C and C: N ratio in conidia. After storage at 30°C the conidia viability decreased with the increase of exposure time, except for the conidia produced in PDA, where the C accumulation and C:N ratio were highest. Also the thermotolerance can be related to the trehalose accumulation, a carbohydrate that can be produced by fungus cultivation in limited nutrient conditions like BDA. These results show that it is possible to manipulate the nutritional conditions in order to obtain an increase in thermotolerance and improve the fungus efficiency in the field.

Poster - Fungi Monday 16:30 **F-04**

Isolation of *Metarhizium* spp. from roots of different crops: Are specific genotypes associated with certain plants?

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Metarhizium brunneum and *M. robertsii*, both formerly belonging to the *M. anisopliae* lineage, have been shown to be associated with the roots of woody plants and wild grasses, respectively. Here we evaluated which *Metarhizium* species were associated with roots of common crop plants in Denmark; oats (*Avena sativa*), rye (*Secale cereale*) and cabbage (*Brassica oleracea*). Thirty-six root samples from each of the three crops were collected within an area of approximately 3 ha. The roots were rinsed with sterile water, homogenized and the homogenate plated onto selective medium. In total, 149 isolates were identified to species by restriction fragment length polymorphism (RFLP) analysis of the gene for elongation factor 1 -alpha (EF1α). Isolates were further genotyped using simple sequence repeat (SSR) analysis of 18 different loci. The roots of all three plant species were primarily associated with *M. brunneum*, which constituted >70% of all isolates, while *M. robertsii* and *M. majus* were only isolated occasionally. The SSR analysis revealed that several genotypes were present within the *M. brunneum* and *M. robertsii* isolates. A single genotype within *M. brunneum* was dominant (>80%). This study further

illustrated that the root of a single plant can host different *Metarhizium* species and genotypes concurrently. The dominant genotype of *M. brunneum* that associated with plants was also isolated most frequently from soil samples of the same field using insect baiting, demonstrating that this genotype is pathogenic to insects.

Poster - Fungi Monday 16:30 **F-05 STU**

Conidial water affinity is an important characteristic for thermotolerance in entomopathogenic fungi

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The thermotolerances of entomopathogenic fungi were evaluated based on their conidial water affinity. Species with hydrophobic conidia were *Beauveria bassiana* (ARSEF 252), *Metarhizium brunneum* (ARSEF 1187), *Metarhizium robertsii* (ARSEF 2575), *Isaria fumosorosea* (ARSEF 3889), and *Metarhizium anisopliae* s.l. (ARSEF 5749). Species with hydrophilic conidia were *Tolypocladium cylindrosporum* (ARSEF 3392), *Tolypocladium inflatum* (ARSEF 4877), *Simplicillium lanosoniveum* (ARSEF 6430), *Lecanicillium aphanocladii* (ARSEF 6433), and *Simplicillium lanosoniveum* (ARSEF 6651). Conidial suspensions were exposed to 45 °C (wet-heat) for 1, 2, 3, 4, 5, and 6 h and then drop-inoculated on Petri dishes (60 × 15 mm) with PDA plus benomyl 0.003%. The conidial germination was evaluated after 24 h for controls and 48 h heat exposed. Species with hydrophobic conidia *B. bassiana* and *M. robertsii* were the most thermotolerant, followed by the other *Metarhizium* species. The species with hydrophilic conidia, *T. inflatum* and *T. cylindrosporum,* had similar medium tolerances. Species with hydrophilic conidia that produce conidia aggregated in balls of slime, i.e. ARSEF 6430, 6433, and 6651, were the least tolerant. *I. fumosorosea* was the only species with hydrophobic conidia that were very susceptible to heat. For the four least thermotolerant isolates (ARSEF 3889, 6430, 6433, and 6651), a survival curve was done at 41 °C with the same time exposure as above. *S. lanosoniveum* was the most thermotolerant at 41 °C, even thought, after four hours exposure, the survival of this isolate was less than 20%. In conclusion, species that produce hydrophobic conidia were more thermotolerant than species with hydrophilic conidia.

We are thankful to the National Council for Scientific and Technological Development (CNPq) of Brazil for grant support 478899/2010-6.

Poster - Fungi Monday 16:30 **F-06 STU**

Tolerance of entomopathogenic fungi to oxidative stress

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Oxidative stress is caused by reactive oxygen species (ROS), including superoxide ion, hydrogen peroxide, and hydroxyl radicals, as well as heat, UV-A radiation, and chemical products. Entomopathogenic fungi are predisposed to heat and UV-A radiation when outside the insect host. When inside the host, they are subjected to phagocytic cells that generate ROS to eliminate invading pathogens. To better understand the oxidative stress tolerance in several fungal entomopathogenic species, we evaluated the resistance of *Beauveria bassiana* (ARSEF 252), *Metarhizium acridum* (ARSEF 324), *Metarhizium brunneum* (ARSEF 1187), *Metarhizium robertsii* (ARSEF 2575), *Tolypocladium cylindrosporum* (ARSEF 3392), *Isaria fumosorosea* (ARSEF 3889), *Tolypocladium inflatum* (ARSEF 4877), *Metarhizium anisopliae* s.l. (ARSEF 5749), *Simplicillium lanosoniveum* (ARSEF 6430), *Lecanicillium aphanocladii* (ARSEF 6433), and *Simplicillium lanosoniveum* (ARSEF 6651) to a strong superoxide-generating agent, menadione sodium bisulphate. The conidial germination was evaluated 24 h after inoculation (maintained at 26 °C in the dark) on PDA media (control) or PDA with 20 different menadione concentrations from 0.01 to 0.20 mM, with increments of 0.01 mM. The two *Tolypocladium* species and the *M. acridum* were the most susceptible fungi. Only *Metarhizium anisopliae* s.l. germinated at 0.20 mM, but this isolate is, the most susceptible to heat and UV-B radiation. The most tolerant isolate to UV-B radiation and heat is *M. acridum*, which was very susceptible to menadione. Among the different *Metarhizium* species high variability in tolerance was found, where *M. acridum* was the least tolerant, *M. robertsii* with medium tolerance, and for *M. anisopliae* with highest tolerance.

We are thankful to the National Council for Scientific and Technological Development (CNPq) of Brazil for grant support 478899/2010-6.

Poster - Fungi Monday 16:30 **F-07**

Antimicrobial and antioxidant activity of culture supernatant of entomopathogenic fungi

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The entomopathogenic fungi are important natural pathogens of insects and have been developed as potential biological control agents for many important agricultural, forest and medical pests. These fungi could produce a wide range of secondary metabolites with high therapeutic value as antibiotics, cytotoxic substances, insecticides, compounds that promote or inhibit growth, attractor and repellent. To investigate the antimicrobial and antioxidant activities, liquid culture filtrates of 347 entomopathogenic fungi isolated form Korea soils were prepared by the quick and easily applicable tool obtaining large number of samples. As results, the supernatant of 72 (20%) and 22 (6%) isolates showed antibacterial activity against *Ralstonia solanacearum* and *Escherichia coli,* respectively, and 22 isolates (6%) had 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals scavenging activity compounds. The preferential antibacterial and radical scavenging activities give evidence that these entomopathogenic fungal metabolites might be useful as a source for bacteria control and pharmaceutical interest.

Poster - Fungi Monday 16:30 **F-08**

The autophagy related gene, *ATG5*, affects conidia yield, morphology, germination and pathogenisis in entomopathogen *Beauveria bassiana*

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Autophagy is a rather conserved degradation pathway that is involved in the maintenance of normal cell differentiation and development. In this study, an autophagy-related gene (*BbATG5*) was cloned and characterized in entomopathogenic fungi *Beauveria bassiana*. This gene had high similarity with the most partners from fungi. Using targeted gene replacement, the gene knockout mutant was generated and fungal autophagy was blocked. Cytological analysis showed that the mutant had abnormal morphological development, including the fluffy mycelium and lower homogeny in conidial shape. Phenotype analysis demonstrated the knockout mutant has dramatic reduction in conidiation and blastospore formation, delayed conidial germination on the starve condition and decreased

virulence against the host. The abnormal phenotypes in mutant were recovered by the introduction of an intact copy of *BbATG5* into the knockout mutant, demonstrating that the *BbATG5* deletion was responsible for the fungal development and pathogenisis defects. These findings suggest that autophagy was involved in cell development and thus essential for the virulence of entomopathogen *B. bassiana*.

Poster - Fungi Monday 16:30 **F-09 STU**

Host-dependent lineage diversification of Scarabaeidae-specific pathogen *Metarhizium majus*

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Metarhizium majus has long been known as a specialist pathogen of Scarabaeidae species and especially known as the biological control agent of a serious coconuts pest, *Oryctes rhinoceros*. Some studies have revealed that each isolate of *M. majus* from different host species has adapted to its own host and has a different host range. However, such intraspecific divergence has not been researched in terms of molecular phylogeny. Phylogenetic relationships between closely related lineages with different host ranges will provide important knowledge about the evolution and taxonomy of entomopathogenic fungi. In this study, we compared phylogenetic relationships and pathogenicity among strains of *M. majus* originating from four genera (three subfamilies) of Scarabaeidae as well as soil. As a result of phylogenetic analysis of the ribosomal DNA intergenic spacer region, strains from different four genera were separated as clades. Four strains from *Oryctes* spp. were clustered in a clade despite their various geographical origins. The host range difference of each clade was confirmed by pathogenicity assay. At least, two pathotypes were recognized. From the molecular dating, the divergence of *M. majus* had occurred far after the subfamilies divergence of Scarabaeidae, suggesting that the emergence of the pathotypes was not the result of host-pathogen co-speciation but the host changes within Scarabaeidae species. Considering its high nutrition requirement of conidial germination, the host changes undergone by the ancestral lineages of *M. majus* may have been restricted to species with chemically similar cuticle.

Poster - Fungi Monday 16:30 **F-10**

Evaluation of two Metarhizium anisopliae for control of drill Paraguay tea Hedypathes betulinus (Klug) adults (Coleoptera: Cerambycidae)

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The drill or tiger of Paraguay tea *Hedypathes betulinus* (Klug, 1825) (Coleoptera: Cerambycidae) is one of the main pests of cultivation Paraguay tea (*Ilex paraguariensis* St. Hil.,1822), which causes severe damages and huge economical loss. It was evaluated under laboratory conditions the pathogenicity of two *Metarhizium anisopliae* CEP 349 and CEP 350, of *H. betulinus* adults. Adult insects of *H. betulinus* were immersed in conidial suspensions of $1x10^8$ conidia/ml. Subsequently, the insects were individualized in plastic containers containing a branch of Paraguay tea and maintained under controlled conditions (26 ± 1 °C, photophase of 14 and 70% relative humidity). The evaluations were done every day for a period of 15 days, determining the percentage of dead insects. The isolates of *M. anisopliae* proved to be pathogenic on *H. betulinus* and mortality values were confirmed over 70%, showing the maximum value in CEP 350 (83.33%) and the minimum value in CEP 349 (70%). Subsequently we determined the median lethal time (LT_{50}) of the population of *H. betulinus* in the range of 7.41 to 7.92 days.

Poster - Fungi Monday 16:30 **F-11**

Identification and phylogenetic analysis of Brazilian strains of *Metarhizium anisopliae s.l.*

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Metarhizium anisopliae s.l. is used in >2 million ha in Brazil annually, mostly against spittlebugs. Strains used for research and as commercial biopesticides in the country, until now have been identified basically based on morphology. However, after the separation of the *Metarhizium anisopliae* lineage into 9 species using a multilocus phylogenetic analysis (Bischoff et al. 2009), some species can only be recognized based on gene sequencing. In this study, we assessed the diversity of 50 *Metarhizium anisopliae s.l.* strains from 13 states, including strains from commercial products, deposited at the Collection of Entomopathogens of the Pathology and Microbial Control Laboratory from the University of São Paulo. We performed partial nucleotide sequencing and a phylogeny analysis of the 5´ end of translation elongation factor 1-alpha gene (EF-1α). Sequence of the type isolates and other were obtained from GenBank to build the phylogenetic tree. Eighty two percent (41 isolates) of the sequences, including all the commercial isolates evaluated (13 isolates), presented 100% similarity with *M. anisopliae s.s.* Only one sequence showed 100% similarity with *M. robertisii*. The others presented 99% or 98% similarity with *M. anisopliae, M. robertisii* or *M. pingshaense.* Only strain ESALQ-1374 did not group to any species.

Poster - Fungi Monday 16:30 **F-12**

Beauveria bassiana* infection alters reproductive parameters of the Chagas disease vector *Triatoma infestans

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Chagas disease is the most important parasitic disease in Latin America, the parasite *Trypanosoma cruzi* is mainly transmitted through blood-feeding triatomine bugs. Current strategies to control *Triatoma infestans*, based on residual chemical insecticide application, are threatened by the emergence of pyrethroid-resistance. Among alternative control tools, we previously showed that the entomopathogenic fungus *Beauveria bassiana* could be used successfully in the field. In this work, we studied the effect of fungal infection on oviposition percentage, number of eggs laid per female, and egg fertility. The trials were carried out with virgin paired males and females. Immediately after copulation, females were exposed to fungi by contact with a fungal powder formulation (*B. bassiana* strain GHA, 2.6×10^8 conidia/cm^2 for 5 min). All parameters were measured daily in both treated and control females. Reproductive capacity was significantly altered ($p < 0.05$) by fungal infection, only 57% of fungus-treated females were able to lay eggs, compared with 100% of controls. The number of eggs per female was significantly reduced ($p < 0.05$) from 35.6 ± 5.4 (control) to 17.3 ± 3.0 (fungus-treated). There were no significant differences on egg fertility between both treatments. As part of a larger research project addressed to understand the complex interactions between entomopathogenic fungi and triatomines, these results will contribute to modeling the population dynamics of indoor *T. infestans* exposed to *B. bassiana* infection.

Poster - Fungi Monday 16:30 **F-13**

Insect cuticular lipid degradation: characterization of cytochrome P450 monooxygenases from the entomopathogenic fungus *Beauveria bassiana*

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Cytochrome P450 monooxygenases constitute a superfamily of heme-thiolate proteins that act on a wide variety of endogenous and xenobiotic molecules. Very long chain hydrocarbons, a major component of the outer insect waxy layer, represent the substrates for a specific subset of these enzymes that participate in hydrocarbon degradation. At least eight cytochrome P450s (CYP) genes potentially involved in lipid metabolism were previously characterized in the entomopathogenic fungus *Beauveria bassiana.* Hydrocarbons and insect cuticular lipids induced the expression of these genes. One *B. bassiana* cytochrome P450 (CYP52X1) was able to oxidize long chain fatty acids. In this work, four additional *B. bassiana* cytochrome P450s: CYP5337A1, CYP52G11, CYP53A26, and CYP584Q, containing C-terminal poly-His tag fusions were expressed in the yeast *Saccharomyces cerevisiae* strain WAT11. After induction, the yeast cells were harvested and endoplasmic reticulum-derived microsomes were obtained. Total membrane proteins were solubilized by treatment with sodium cholate, and loaded onto columns containing nickel-charged resin for purification of the His-tagged proteins. The molecular masses of the purified proteins were estimated by SDS-PAGE and total cytochrome P450 content was measured by spectrophotometry. These results, together with ongoing experiments on substrate specificity, will help characterize the biochemical properties of the cytochrome P450s of *B. bassiana* leading to better understanding their roles in insect lipid degradation.

Poster - Fungi Monday 16:30 **F-14**

Root colonizer and endophytic abilities of entomopathogenic *Lecanicillium* spp.

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There has been little unequivocal evidence of true rhizosphere competence (growth of the fungus within the root zone utilizing plant carbon) in entomopathogenic fungi. The mechanisms of interaction between fungus and plant root needs to be elucidated. Gaining an understanding of the population structure of rhizosphere colonizers and how they change throughout the season is imperative for development of strategies for controlling plant parasitic nematodes, root diseases and improving root health. In this experiment, we analyzed the abilities of root colonizer and endophyte of *Lecanicillium* muscarium and hybrid strains (*L. muscarium* × *longisporum*) using tomato, cucumber and melon plants. Finally, we founded that several strains could be good root colonizer and endophyte.

Poster - Fungi Monday 16:30 **F-15 STU**

Comparing pathogenicity and infectivity of anamorphic entomopathogenic fungi isolated from the whole or inside of wild mosquito body against adult female *Anopheles stephensi*

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Our previous study suggested that wild adult mosquitoes have various entomopathogenic fungi that might have the potential for controlling vector mosquito. So, the aim of this study was the comparison of pathogenicity and infectivity of anamorphic entomopathogenic fungi isolated from whole mosquito body (body isolates) and isolates originated from inside of the mosquito body (inside isolates). 36 body isolates and 23 inside isolates were applied to evaluation of pathogenicity and infectivity against adult female *Anopheles stephensi*. Although, only 28 body isolates showed infectivity to adult mosquito, all inside isolates had infectivity to them. However, 23 inside isolates tended to have low pathogenicity. Among them, 24 isolates (12 body isolates and 12 inside isolates) which showed higher infectivity or pathogenicity were applied for more three replications to estimate the 50 % lethal time (LT_{50}). As a result, LT_{50} values were ranged from 5.8 days to 12.5 days in body isolates, and 6.6 days to 14.9 days in inside isolates (control value was 16.6 days). Especially, *Beauveria bassiana* 60-2 showed highest virulence and infection rate against *An. Stephensi* (5.8 days and 82 % respectively). Furthermore, several isolates that showed infectivity but had low virulence were detected. These results suggested that although, even wild mosquitoes having flight capability infected entomopathogenic fungi, their virulence were low. And high virulence isolate might affect flight capability, so these isolates were isolated from integument of the mosquito body before establishment of infection.

Poster - Fungi Monday 16:30 **F-16**

Effect of temperature on radial growth of *Beauveria bassiana* and *Metarhizium anisopliae* isolates pathogenic to boll weevil, *Anthonomus grandis*.

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Temperature is one of the environmental factors that influence fungal growth and disease development in insects. Selection of fungal pathogens tolerant to the temperature range found in the agricultural ecosystem involved is essential in order to use insect pathogens in IPM programs. The aim of the present study was to evaluate the effect of the temperature on radial growth of two *B. bassiana* and one *M anisopliae* isolates selected for boll weevil control. Aliquots of 5 µL of conidial suspension (10^5 conidia/ml) for each isolate were inoculated on the centre of each plate. Cultures were incubated at 23, 27, 30, 35 and 38 ± 1ºC with six replicate plates per temperature and isolate. Radial growth was recorded daily for 15 days. The growth rates (mm/day) at different temperatures were calculated from the regression slope of colony diameter versus time during the linear growth phase, and were compared by ANOVA. The highest growth rate was at 27ºC for all isolates. At 30 and 35ºC, the *M. anisopliae* isolate showed a higher growth rate than *B. bassiana.* All the tested isolates were inhibited at 38ºC. These results indicate that the *M. anisopliae* isolate could be appropriate for management of *A. grandis*, due to its higher temperature tolerance. However, it is necessary to study if the temperature affects the virulence of the isolate and the disease develops successfully in field conditions.

Poster - Fungi Monday 16:30 **F-17**

Preliminary study in the selection of *Metarhizium anisopliae* isolates for microbial control of "stable fly" (*Stomoxys calcitrans*) and "house fly" (*Musca domestica*) in dairy

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The use of the entomopathogenic fungus *Metarhizium anisopliae* (Ma) as a potential agent of microbial control against "stable fly" (*Stomoxys calcitrans*) and "House Fly" (*Musca domestica*) and its future application in an Integrated Pest Management Plan in dairies were considered. Screening bioassays were performed where 14 isolates of Ma against Stable fly (SF) and 18 isolates against House Fly (HF) were tested. All isolates belong to the collection of the

Entomopathogenic Fungi Laboratory (IMyZA) in INTA Castelar. 6-days larvae in SF bioassays and 2-days larvae in HF bioassays were inoculated using the immersion method in a conidial suspension of $5x10^8$ conidia viable/ml. There were 5 replicates with 10 flies each per treatment. The controls were treated with water solution containing 0,05% of tween 80. The number of dead flies was recorded during 15 days and percent mortality (%M) caused by fungi infection was calculated for each isolate. The isolates that presented %M more than 80% were selected. The isolates Ma49, Ma24 and Ma20 with %M: 80±16, 82±13 and 90±11 respectively were selected for SF. The isolates selected for HF were Ma45, Ma46, Ma47; Ma48 and Ma49 with %M: 88±11, 88±13, 84±6, 88±9, 82±15 respectively. This study allows to continue in the development of a micoinsecticide with the preselected isolates, where the more virulent isolates may be included in a biocontrol plan of the pest in diaries.

Poster - Fungi Monday 16:30 **F-18**

Field applications of entomopathogenic fungi to control *Diaphorina citri* (Hemiptera: Psyllidae) in Brazil

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The citrus greening disease also known as Huanglongbing, poses a serious threat to citrus production in Brazil. This disease is vectored by *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). The aim of this study was to evaluate the efficacy of two entomopathogenic fungi *Isaria fumosorosea* and *Beauveria bassiana* to control of *D. citri* adults in semi-field and field conditions. Twenty adults were confined in each plant using "voile" bags and spraying carried out by the voile. Semi-field trials were conducted in citrus plants of 1 meter tall in Piracicaba, São Paulo. Pulverization was carried out at 2.5; 5 and 10 x 10^6 conidia/ml of *B. bassiana* and *I. fumosorosea* and the experiment was repeated once. After ten days, total mortalities were higher than 94% in the first trial and higher than 79% in second trial for all treatments. Mortality of the control treatment, where insects were sprayed with only water, was 6.6% and 1.8%, respectively. Field assays were conducted in commercial citrus groves in Itapetininga, São Paulo, Brazil confining 20 adults per "voile" bags per plant. *B. bassiana* and *I. fumosorosea* suspensions were applied three times, at a rate of 1 x 10^{12} conidia/ha in 1000 L or 2000 L, using a tractor-mounted turbo sprayer. Average *D. citri* mortalities of the three trials were 78.4% and 75,6% for *B. bassiana* and 83.2% and 89.8% for *I. fumosorosea* for sprays with 1000 L/ha and 2000 L/ha, respectively. The control mortality was 23.6% and treatments with chemical insecticide resulted in 90% mortality. These results suggest that these entomopathogenic fungi may be promissory bioinsecticide for the control of *D. citri.*

Poster - Fungi Monday 16:30 **F-19**

Occurrence and distribution of insect pathogenic soil fungi in agro and forest ecosystem in Eastern Georgia

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Entomopathogenic fungi naturally occuring in the soil represent a reservoir of antagonists to insect pest. Local strains of such fungi may be adapted to their environment and therefore they are of particular interest for usage in biological control. Georgia has a high diversity concerning altitudes, eco-systems and cropping systems, offering special opportunities for studies of insect pathogens. Soil samples were obtained in 2010-2011, from four different sites at different altitudes (600-1700 m a.s.l), representing different agro- and forest ecosystems of Eastern Georgia. A total of 48 soil samples representing 16 locations, were analysed using the insect bait method (Waxworm, *Galleria mellonella* L. and Mealworm*, Tenebrio molitor*). The following entomopathogenic fungal taxa were found: *Beauveria bassiana s.l, Beauveria brongniatii, Metarhizium spp., Lecanicillum sp.* Also, we isolated *Aspergillus flavus.* The most abundant species was *Beauveria bassiana* (61.9 % of samples). Three isolates of both *Metarhizium spp.* and *Lecanicillum sp*. were found, while only one *Beauveria brongniatii.* Interestingly, no entomopathogenous fungi were isolated from six of the soil samples. In these locations, *B. bassiana* was predominantly recovered more often from soils of natural habitats, while *Metarhizium spp.* was recovered mostly in agricultural habitats. Our study included a limited number of samples and more extended studies may reveal additional information about occurrence of these fungi in different habitats. This research was funded by STCU-SRNSF, Grant # 5253.

POSTER SESSION 1 Monday, 16:30 – 18:30

MICROBIAL CONTROL

Poster – Microbial Control Monday 16:30 **MC-01**

Study on the characteristics and pathogenicity of *Beauveria bassiana* as a control agents of *Hyphantria cunea* (Lepidoptera: Arctiidae)

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Strategies for control of the Fall webworm, *Hyphantria cunea* (Lepidoptera: Arctiidae), include the use of entomopathogenic fungi. In 2010 and 2011 several isolates of *Beauveria bassiana* were isolated from several agro-ecosystems in East Georgia. Three of these isolates were evaluated for key physiological characteristics, as well as pathogenicity, to determine the most effective one for possible use against this insect. Mycelial growth rate and sporulation yield of three isolates were different from each other after 20 days on Sabouraud dextrose yeast agar; isolate Bb025 had the greatest mycelial growth rate and conidial yield, followed by the isolates Bb007 and Bb011. Mycelial growth rate was 3.75, 3.22, 2.85 mm/day, and conidial production was 21.15, 18.10 and 17,35 x 10 conidia/cm^2, for the three respective isolates. Bioassays used three different spore concentrations (10^6, 10^7, and 10^8 conidia ml^{-1}) of Bb025, against 4th and 5th instars *H. cunea* larvae*;* dosing was by immersion of insects in conidial suspensions. The larvae were then reared in glass jars with tree leaves as food, at 23±2 ºC, 45-65% RH, and 16:8 light:dark photoperiod. Larvae were checked daily to detect symptomatic or dead larvae, which were removed and placed individually in Petri plates with high moisture environment for development of sporulating mycelium to verify infection. Efficacy of Bb025 was 47,5, 66,7 and 72,3% for the three doses 12 days after treatment. This research was funded by STCU-SRNSF, grant # 5253.

Poster – Microbial Control Monday 16:30 **MC-02 STU**

Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* to the legume pod borer, *Maruca vitrata* and the performance of two candidate isolates in four liquid culture media

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The Legume pod borer, *Maruca vitrata* is one of the most damaging insect pests of cowpea causing yield losses of 20-80% in sub-Saharan Africa. We screened 20 isolates of *Metarhizium*

anisopliae and *Beauveria bassiana* against first instar larvae with the aim of identifying and selecting the most virulent isolates for managing the pest. The larvae were exposed to fresh cowpea flowers treated with aqueous suspension of the fungal isolates at a concentration of $1x10^8$ conidia ml^{-1}. Larval mortality ranged from 10-91% mortality at 7 d post inoculation. Two isolates of *M. anisopliae* (ICIPE 18 and ICIPE 69) outperformed the rest, causing 91.2 ± 5.1% and 80.9 ± 9.1% mortality with LT_{50} values of 1.6 and 1.1 days, respectively. Growth and propagule production of the 2 isolates varied across 4 liquid media (Ademek, Jenkin-Prior, APU1 and APU2) tested for mass production. Maximum production of dry weight was observed after 48 h in ICIPE 69 (68.3 ± 5.2 mg^{-1} suspension) and after 72 h (41.6 ± 3.1 mg^{-1} suspension) in ICIPE 18. After 72 h, ICIPE 69 produced higher concentration of propagules in Jenkin-Prior (2.6 ± 0.4 x 10^8 propagules ml^{-1}) and APU1 (2.4 ± 0.7 x 10^8 propagules ml^{-1}) media compared with ICIPE 18 (Jenkin-Prior: 1.5 ± 0.2 x 10^6 propagules ml^{-1}; APU1: 5.2 ± 1.7 x 10^7 propagules ml^{-1}). In both isolates the lowest propagule biomass and concentration were observed in APU1 medium. Our results demonstrate that ICIPE 69 has high virulence and better production characteristics critical for a candidate biopesticide.

Poster – Microbial Control Monday 16:30 **MC-03**

Preliminary study of *Metarhizium anisopliae* production on bioreactor: concentration of inoculum

Vivian Amanda F. Costa[1], Fabrício M. Buriola[1], Adriana Regina Generoso[3], Mariana Taglietto de Oliveira[2] and Cesar de O. Guimarães[4]

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With the growth of sugarcane crop, biological control is expanded to provide a cheaper and less environmentally aggressive alternative to pesticides. Despite increase in commercial use of biological control and especially in the entomopathogenic fungus *Metarhizium anisopliae*, its production still uses traditional and inefficient techniques. This reveals the urgent need to improve the sector to ensure a product in quantity and quality necessary for their commercial use in agriculture. This work is part of a broader project whose objective is to produce *M. anisopliae* in a bioreactor. For this, several preliminary bioassays are necessary to optimize the growing of the fungus in the bioreactor. One of these is evaluate the best amount of inoculum. The goal of this study was to determine the best ratio between concentration and volume inoculum to be used in the production of *M. anisopliae* and ensures a quality production with minimum use of matrices ensuring low production costs. For this, the fungus was inoculated by several combinations of concentration and volume, in rice type 1 and after 15 days incubation the conidial production was evaluated. It was observed that only the variation of volume used influenced the amount of conidia produced with the highest value observed when we used 1 ml of inoculum. In tested parameters, the concentration showed no significant differences. It's possible conclude that the ratios of 0,03 ml of inoculum per gram of rice, with a concentration of about 10^4 viable conidia enables the best conidial production among tested conditions.

Poster – Microbial Control Monday 16:30 **MC-04**

Influence of treatment interval between eco-friendly agricultural products and *Beauveria bassiana* GHA for sweetpotato whitefly control

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Various pesticides may be applied to control insect pests and plant disease. Pesticides, especially fungicides can reduce viability of microbial control agents such as fungal spores and may decrease control efficacy of mycopesticides. When diverse pesticides are used in a crop, their compatibility with other control agents and application timing is important. We conducted a study of the safe treatment period between the mycopesticide *Beauveria bassiana* GHA and eco-friendly agricultural materials used for pest or disease control in Korean egg plant production. Among fungicides tested, the mixture of Japanese apricot + ginkgo nut extracts and the wettable sulphur powder required an interval of 7 days. A mixture of Japanese honeysuckle + jeffer sonia + Korean pasque flower extracts required 4 day intervals to obtain similar control efficacy with *B. bassiana* GHA treatment alone. Among insecticides tested, the extract of yellow sophora and the mixture of yellow sophora + derris required a 4 day intervals. The other commercial extract of yellow sophora and the mixture of bead tree + yellow sophora didn't require any application intervals to control sweetpotato whitefly.

Poster – Microbial Control Monday 16:30 **MC-05**

First record of the genus *Protomagalhaensia* (Eugregarinida: Hirmocystidae) Pinto, 1918 in cockroaches from Argentina.

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Parasites and pathogens of cockroaches from the Neotropical region are poorly known. During a survey of natural enemies of cockroaches from La Plata city, Argentina, we found nymphs and adults of *Blaptica dubia* Serville, 1839 (Blattodea: Blaberidae) infected with gregarines. Specimens were dissected and their digestive tube examined under a compound microscope with phase contrast. Gamonts were observed in the gastric caeca and the descending ventriculus. Measurements and characteristics of the trophozoite epimerite, gamont protomerite and the caudofrontal association, also the gametocyst dehiscence by simple rupture, and the oocysts characteristics place this gregarine into the genus *Protomagalhaensia*, Order Eugregarinida, Suborder Septatina, Family Hirmocystidae. DNA from each pooled sample was maintained in 70% alcohol and stored at -20ºC for future molecular analysis. This genus is cited for the first time in this cockroach in Argentina.

Poster – Microbial Control Monday 16:30 **MC-06**

Detection and imaging of Metarhizium infection of wireworms using antibodies and electron microscopy

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We have taken preliminary steps to develop serological techniques and scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) to detect Metarhizium infections of wireworms and have had good success in two crucial areas: i) developing a polyclonal antibody for Metarhizium; and ii) developing both SEM and CLSM techniques, particularly techniques for specimen handling and preparation. Using CLSM, we have imaged mycelia and conidia on the cuticle and mycelia in the haemocoel. The application of antibodies bound to Metarhizium yields excellent images, as presented. We aim to use these imaging techniques as a tool to observe and further investigate aspects of histopathology between Metarhizium and wireworms, and other insect-pathogen combinations as well.

Poster – Microbial Control Monday 16:30 **MC-07**

Screening and evaluation of entomopathogenic fungi to the green peach aphid, *Myzus persicae*

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The green peach aphid, *Myzus persicae* Sulzer, is an economically important pest for greenhouse crops, because they cause direct damage by feeding on plant nutrients and indirect damage as transmits many virus vectors. Moreover, the overuse of insecticide has resulting in resistance among green peach aphid population and environmental pollution. Thus, the development of entomopathogenic fungi has received increasing interest as part of integrated pest management strategies as aphid biocontrol agents. In this study, we report the screening result of pathogenic fungi for the control of green peach aphid. Initial screenings were performed using 347 isolates of putative pathogenic fungi from Korea soils. As results, 20 isolates of entomopathogenic fungi were isolated from cadavers of green peach aphid supporting fungal conidiation. These isolates were identified as three strains of *Lecanicillium attenuatum*, nine strains of *Beauveria bassiana*, one strain of *Metarhizium anisopliae*, one strain of *Metarhizium flavoviride*, five strains of *Paecilomyces lilacinus*, one strain of *Aspergillus* sp. by microscopic examination, genetic sequencing of the ITS region and Universally Primed PCR (UP-PCR). Based on the screening results, twenty isolates were tested for their pathogenicity against adult green peach aphid, cold activity, thermotolerance, UV-B irradiation on conidia. All fungal isolates were pathogenic to green peach aphid but mortality varied with isolates. These entomopathogenic fungi may be useful to develop eco-friendly insecticide to control green peach aphid.

Poster – Microbial Control Monday 16:30 **MC-08**

Efficacy of entomopathogenic hypocrealean fungi to *Periplaneta americana*

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Periplaneta americana is one of the world's most important synanthropic cockroaches and causes severe allergies and mechanically spreads pathogenic microbes in hospitals and restaurants. Increasing resistance to chemicals emphasizes the need to develop new biorational insecticides. Because so little is known about the potential of entomopathogenic fungi to control *P. americana* we investigated the pathogenicity of eleven species from seven entomopathogenic ascomycete genera against nymphs and, the efficacy of selected fungi against oothecae and adults. *B. bassiana*, *M. anisopliae* and *M. robertsii* were pathogenic to *P. americana* (≥ 81.7% mortality, 20 days p.i.), but not *Isaria cateniobliqua*, *Isaria farinosa*, *M. frigidum*, *Purpureocillium lilacinum*, *Simplicillium lanosoniveum*, *Sporothrix insectorum*, and *Tolypocladium cylindrosporum* (≤ 20% mortality, 20 days p.i.). Susceptibility of adults to *M. anisopliae* was dose-dependent and did not differ between sexes. Grooming behavior of nymphs and adults immediately after fungal application eventually reduced quantitative exposure to conidia and fungal effectiveness. Oothecae were clearly less affected by *M. anisopliae* and *M. robertsii* than nymphs. A total of 31% and 23% of oothecae treated with *M. anisopliae* or *M. robertsii*, respectively, had visibly shrunk and were covered with mycelium and conidia; no nymphs eclosed from these oothecae. Nymphs that eclosed successfully from oothecae did not succumb to fungal infection. The results found in this study made clear that *Metarhizium* spp show especially strong potential as antagonists of *P. americana*. Future efforts against this important pest will focus on strains of both genera *Beauveria* and *Metarhizium* and specific formulation and application techniques.

Poster – Microbial Control Monday 16:30 **MC-09**

A new formulation of *Metarhizium anisopliae* against *Triatoma infestans*

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Dry conditions in triatomine microhabitats appear to be a major drawback to developing effective mycoinsecticides against the vectors of Chagas disease in Latin America. Reports on emerging insecticide-resistant domestic *Triatoma infestans* populations are alarming, and other species eventually invade houses from peridomestic or sylvatic areas. Triatomines are highly susceptible to fungal infections at high humidities but mortality declines at drier conditions. Oil-formulated conidia did not provide distinctly higher mortalities against these vectors. The present study reports on a new formulation of *M. anisopliae* combining oil and diatomaceous earth (DE) against *T. infestans*. Eggs and third instar nymphs (N3) were exposed on filter paper pretreated with *M. anisopliae* IP 46 conidia (10^7 conidia/cm^2) formulated in oil (3 μl/cm^2), DE (1 mg/cm^2) or with both additives in combination, and incubated at 25°C and 75% relative humidity (RH) or close to saturation (RH >97%). Eggs and nymphs were highly susceptible to infection with *M. anisopliae* when incubated in humidity close to saturation, regardless of the formulation tested. In 75% RH, eggs were resistant to infection, with 90% eclosion of nymphs. In contrast a total of 86.9% of eclosing nymphs (N1) died within 25 days when kept in contact with the filter paper treated with a combined conidial/DE formulation. No N3 survived within 15 days of exposure when tested at the same conditions. The combined *M. anisopliae* formulation was highly effective even in suboptimal humidity, and promises to accelerate development of a mycoinsecticide against triatomine vectors.

Poster – Microbial Control Monday 16:30 **MC-10**

Decreased viability of the hemocytes of *Galleria mellonella* larvae under the *Habrobracon hebetor* venom.

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The cytosolic calcium concentration in the hemocytes of *Galleria mellonella* larvae was measured on first and second day after envenomation by *Habrobracon hebetor* female. Changes in level of the free cytosolic Ca^{2+} in blood cells have been registered by fluorescence marker (fura - 2 AM). The increase in Ca^{2+} concentration about three times was noted on the first day. On the second day the concentration of cytosolic calcium was exceeded control values only to 1.5 times. Simultaneously, we measured the activity of phospholipase C. The significant increase in the enzyme activity was noted for two days after injection. The effect of the parasitoid venom on the viability of blood cells was carried out *in vitro* by trypan blue. We were detected decrease in the viability of hemocytes within one hour after the addition of venom (final concentration of venom 0.0062 mg/ml). In addition we was recorded the reduced adhesive capacity of the hemocytes for hour after the addition of venom (final concentration of venom 0.003 mg/ml). In addition, we measured the membrane potential. As a fluorescent probe, we applied 4-(4-dimethyl-aminostyryl)-1-methylpyridinium, DSM, which is potential

sensitive and used for determination of potential of cell and mitochondrial membranes. We tested the change of trans-membrane potential of hemocytes both *in vitro* and *in vivo* experiments. The degree of decreasing was in direct dependence of the venom concentration. The paralyzed insects exhibited the decreased potential values.

Poster – Microbial Control Monday 16:30 **MC-11**

Metarhizium anisopliae* for the control of *Aedes aegypti

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Expectations about using entomopathogenic fungi to control aedine and other mosquitoes are high. Increasing the efficacy of mycoinsecticides will depend on improving formulation and application technologies adapted to the target vector. *Metarhizium anisopliae* can eliminate *Aedes aegypti* eggs, larvae and adults, and applications of *M. anisopliae* in domestic areas where gravid females and their offspring concentrate can reduce populations of this vector. In laboratory tests, females died from mycoses and the fungus sporulated on cadavers after as little as a 60 second exposure time to a surface treated with oil- formulated *M. anisopliae* conidia (0,25 µl/cm^2). In choice tests, gravid females oviposited on moistened filter paper previously treated with oil-in-water formulated conidia and were obviously not repelled by the fungus. Mycelium and conidia developing subsequently on eggs seemed to stimulate larval eclosion on the filter paper even in the absence of water. When treated eggs were held at 25°C and humidity >98% for 15 days and then immersed in water, eclosion decreased higher conidial concentrations. The concentration inhibiting 50% eclosion (compared to the control) was 4.9 x 10^3 conidia/cm^2 treated surface. First field tests in the city of Goiania, Brazil, in 2010 and 2011 with simple fungus-treated breeding devices showed the activity of *M. anisopliae* against eggs laid by aedine females and confirmed results found in laboratory assays. A better performance of devices will improve their effectiveness in the field and establish a valid prospect for mycoinsecticides for integrated control of aedine mosquitoes.

Poster – Microbial Control Monday 16:30 **MC-12**

Virulence of Brazilian isolates of entomopathogenic fungi against different life stages of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae)

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Virulence of 14 isolates of *Beauveria bassiana*, *Isaria fumosorosea* and *Lecanicillium* spp. from Brazil was determined against eggs, 2nd instar nymphs and adults of *Bemisia tabaci* biotype B on bean leaves under laboratory conditions. Isolates of *B. bassiana* and *I. fumosorosea* were the most virulent against nymphs presenting high mortality rates (71-87% within 7d) and LT_{50} ranging from 3.25 to 4.25d, after direct spray of 150 conidia/mm^2 (10^7 conidia/mL). Sporulation from nymph cadavers killed by two isolates of *B. bassiana* reached the highest yield (8×10^5 conidia/cadaver). *Lecanicillium* isolates were not tested in subsequent trials, since they showed low virulence to nymphs. Very low infection was observed on eggs sprayed with *B. bassiana* and *I. fumosorosea* at 1674 conidia/mm^2 (10^8 conidia/mL); however, nymphs were highly susceptible to some isolates of these fungi by contamination on sprayed leaves soon after egg hatching, due to residual effect. Indirect effects of the fungi were tested by exposing adults to treated leaf discs (1674 conidia/mm^2). After 8d exposure, 98-100% adults were infected by *I. fumosorosea* isolates, whereas *B. bassiana* resulted in 33-81% infection. Additionally, we assessed the conidial production of these two fungi in parboiled rice solid-state fermentation. After 10-11d incubation at 26ºC and 14 h light, high conidial yields (4.9-10.8×10^9 conidia/g) were observed by one isolate of each fungus. The most virulent Brazilian isolates will be used to develop an effective mycoinsecticide against *Bemisia tabaci* biotype B.

Poster – Microbial Control Monday 16:30 **MC-13**

Enhanced susceptibility of *Tibraca limbativentris* (Heteroptera: Pentatomidae) to *Metarhizium anisopliae* with sublethal doses of chemical insecticides

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In this study was investigated the interaction between the fungus *Metarhizium anisopliae* with sublethal doses of synthetic chemical insecticides Actara™ (thiamethoxam) and Karate Zeon™ (lambda-cyhalothrin) for the control of *Tibraca limbativentris* adults under laboratory and field conditions. Time to death was reduced significantly when *M. anisopliae* at 5×10^6, 5×10^7 and 5×10^8 conidia/mL was combined with 0.78 ppm (AI) of thiamethoxam or with 2.5 ppm (AI) of lambda-cyhalothrin. Additionally, the thiamethoxam-fungus combination increased overall mortality and percent mycosed insects in comparison with each component alone, except for lambda-cyhalothrin that did not improve mycosed insects. The increase of fungus concentration did not significantly improve insect mortality when combined with doses of thiamethoxam <0.78 ppm (AI). The most feasible combination was the fungus at 5×10^6 conidia/mL with 0.78 ppm (AI) of thiamethoxam. In the field experiment, the combination of *M. anisopliae* at 1×10^{12} viable conidia/ha with thiamethoxam at 12.5 g (AI)/ha (¼ full dose), increased synergistically mortality and mycosis of adults of *T. limbativentris.* The strategy of using sublethal doses of chemical insecticides in combination with entomopathogenic fungi consists of an effective and feasible control method to battle the rice stink bug in the field from an integrated and sustainable pest management viewpoint.

Poster – Microbial Control Monday 16:30 **MC-14**

Development and testing of formulations of *Lecanicillium* spp. for the biological control of white flies.

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The entomopathogenic fungus, *Lecanicillium lecanii* is a biocontrol agent with a wide host range. It has been reported as effective for the control of homopteran insects, mainly aphids, whiteflies, scale insects and thrips, although it has also been isolated from insects, spiders and mites. In addition, *L. lecanii* can be a mycoparasite of rust and other phytopathogenic fungi, such as powdery mildew. In this study, laboratory experiments were conducted to select *Lecanicillium* spp. strains and formulations for use in tomato glasshouse crops against the white fly, *Trialeurodes vaporariorum*. Production media, including standard media and molasses yeast broth (MYB) of different C:N ratios, were evaluated. Highest conidial concentration (1.7 x 10^9 blastospore/ml), biomass yield (15 mg/ml) and germination rate (89.4 %) were obtained using MYB with a 1:1 C:N ratio. Wettable powder and pellet formulations were prepared with fungal blastospores and pathogenicity was

evaluated against *T. vaporariorum* nymphs using a detached leaf bioassay. Mortality was first observed 4 days after spraying 1 ml of the formulation (1.0 x 10^6blastospore/ml) and reached infection levels >90% by day 7 after treatment. Survival of formulated blastospores in storage at 5ºC and 20ºC was assessed. After 3 weeks' storage, pellet formulations maintained 62% viability at room temperature and 74% in refrigerated conditions, meanwhile powder formulations retained about 15 and 57% respectively. Further studies to improve blastospore viability are being carried out.

Poster – Microbial Control Monday 16:30 **MC-15**

Combined use of *Steinernema brazilense* with *Beauveria bassiana* against the sugarcane billbug, *Sphenophorus levis* (Coleoptera: Curculionidae)

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Sphenophorus levis is considered one of the main pests of sugarcane crops in São Paulo state, where this crop is most explored in Brazil. This study evaluated the combined use of Steinernema brazilense IBCB n6 + Beauveria bassiana IBCB 66 against 3^{th}- 4^{th} instars larvae (25 days age) of *S. levis*. The fungus and the nematodes were obtained from the Entomopathogens Collection of the Instituto Biológico, Campinas, SP. The insects were obtained from the Entomology Laboratory rearing that belongs to the Centro de Tecnologia Canavieira (CTC), Piracicaba, SP. Billbug larvae were artificially infested in sugarcane stalks of 8.0 cm length as following: one role was made in the rhizome of each stalk and the larva was inserted using one larva for each role. The stalks were planted in pots with 800 g of sandy soil moistened to 10%. Four treatments were considered as following: 1) S. brazilense + Tween® 0,1% sprayed on the soil at the dose of 3.0 IJs/cm^2; 2) B. bassiana + Tween® 0,1% sprayed on the soil at the volume of 5 mL containing 1,0 x 10^8 conidia ml^{-1}; 3) Combination of both agent + Tween® 0,1%; and 4) Control (water + Tween® 0,1%). The larvae mortality was assessed 7 days after application. The fungus provide 3,3% mortality, while the nematode provided 60% and the combined agents, 80%. Although the combined agents provided the higher mortality, this treatment did not differed statistically from the nematode alone treatment. Only the fungus treatment did not differ statistically from control.

Poster – Microbial Control Monday 16:30 **MC-16**

Preliminary study of *Metarhizium anisopliae* production on bioreactor: use of inert with rice as substract

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Biological control has reemerged as an important alternative to pesticides, and in some sectors, is already a strong competitor to chemical pesticides not only due to the lower cost but also less environmental impact. Commercial production of *Metarhizium anisopliae*, despite the important economic activity, has needed to modernize their processes that are highly handmade. This work is part of a broader project whose objective is to produce *M. anisopliae* in a bioreactor. For this, several preliminary bioassays are necessary to optimize the growing of the fungus in the bioreactor. One of these is decrease the aggregation of rice inside the bioreactor. Thus, the purpose of this study was to determine whether the addition of crushed husk rice may enhance the production of conidia of the fungus *M. anisopliae* by improving the physical characteristics of the bed fermentation. For this crushed husk rice was added to rice type 1 (substrate) in various combinations of size and quantity and then autoclaved. This mixture was inoculated with a spore suspension. After 15 days of incubation the conidial production was evaluated. It was observed that there is a tendency for higher production of conidia when husk rice was added. Moreover, it was noted ease of manipulation during the homogenization of the substrate after inoculation. The promising aspect of the results suggests that further studies are more comprehensive than can establish the optimal proportions of husk and substrate as well as test other types of inert that can be used in commercial production.

Poster – Microbial Control Monday 16:30 **MC-17**

Insecticidal potential of new *Bacillus thuringiensis* and *Lysinibacillus sphaericus* strains against *Spodoptera frugiperda* (Lep. Noctuidae).

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The fall armyworm, *Spodoptera frugiperda*, is a very important pest that attacks various cultures causing significant production losses. In this way, the present research aims to evaluate insecticidal potential of new strains of *Bacillus thuringiensis* and *Lysinibacillus sphaericus* against third instar *S. frugiperda* larva. Ten strains of each entomopathogenic bacteria were selected from the Unisinos/BBE collection. The strains were grown glucosed medium at 28°C, 180rpm and 48 hours, the suspension were centrifuged, the pellet was washed in phosphate buffer and the concentrations adjusted from 1.1010 cell/mL. Bioassays consisted of 20 insects by treatment and the control, which suspension was replaced with sterile distilled water. The mortality analysis was performed seven days after treatment application. The corrected mortality (CM) was calculated using Abbott's formula. The results showed that for *B. thuringiensis*, strain *Bt* 3420-11 was the highest mortality, causing CM of 88%. For *L. sphaericus*, strain *Ls* 1867-4 was the highest mortality, causing only CM 31%. From the tested isolates, no *L. sphaericus* strains showed any relevant insecticidal activity to *S. frugiperda.* The isolate of *B. thuringiensis Bt* 3420-11, can be assessed through purifying Cry proteins to confirm their toxicity against *S. frugiperda* larva.

Poster – Microbial Control Monday 16:30 **MC-18**

Toxicity of transgenic indica *Bt*-rice (IRGA-424), expressing Cry1Aa toxin from *Bacillus thuringiensis* to *Spodoptera frugiperda* (Lepidoptera: Noctuidae), in laboratory.

Laura Massochin Nunes Pinto[1], Caroline Agriardi[1], Fernanda Pavani[1], Shana Wiest[1], Jaime Oliveira[2], Valmir Menezes[2], Athos Gadea[2], Maurício Fischer[2], Pascal Gantet[3], Emmanuel Guiderdoni[3] and Lidia Mariana Fiuza[1,2].

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Rice is the basic food for over 50% of the world's population, but rice crops lose productivity due to incidence of insect pests. The rice expression of *cry* genes, from *Bacillus thuringiensis* (*Bt*), that codes insecticidal proteins, has the potential to reduce this damage without causing environmental harm as chemical insecticides. This study aimed to perform a resistance evaluation of indica rice cultivar IRGA 424 transformed with the *cry1Aa* gene from *Bt*, in order to obtain rice plants resistant to *Spodoptera frugiperda* larvae. The detached-leaf bioassays were carried using terminal stem leaves of rice which were cut into 2

cm sections from each plant per transgenic line and the control (non transformed IRGA 424). Each leaf section was placed on 30mm-diameter plastic dishes with filter paper moisturized with sterilized distilled water. The *S. frugiperda* neonate larvae (12~24h old) were transferred to each plastic dish and placed individually. Thirty larvae were evaluated for each one of the 4 transgenic lines, with three replicates, totalizing 450 evaluated insects. The bioassays were carried out in B.O.D. chambers at 25± 2°C, 80% R.H. and 12 hours photophase. Larval mortality was recorded until the seventh day after treatments and the data was corrected using Abbott's formula. In this work, all the 4 *Bt*-rice plants caused high mortality to *S. frugiperda*. The results showed a corrected mortality from 65 to 84% to the target insect. These laboratory studies indicated that these new Brazilian *Bt*-rice strains might be able to effectively control the fall armyworm, *S. frugiperda*.

Poster – Microbial Control Monday 16:30 **MC-19**

New *B. thuringiensis* isolates with high toxic activity against Lepidopteran larvae in Mexico.

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The use of biopesticides in Mexico is limited by manufacturing technology, because the commercially available products are imported and expensive compared to chemicals. Moreover, the search for new strains of *Bacillus thuringiensis* continues, as new strains are being isolated with different cry genes that can potentially produce new activities. Within this objective were isolated 80 new strains of *B. thuringiensis*, of which 2 were selected in preliminary bioassays. These strains were cultured in soybean meal, corn steep liquor, molasses and minerals for three days at 30 ° C and 200 rpm agitation. After extracting the insecticidal complex composed by spores and crystals, it was evaluated against neonate larvae of *Trichoplusia ni, Heliothis virescens* and *Spodoptera frugiperda* by using an artificial diet on bioassays. Insecticidal extracts of these strains were compared against the extract produced by the strain GM-7 (reference strain highly toxic). The average values of LC_{50} obtained were 178, 147 and 278 ng/cm^2 for new strains GM-IB-7, GM-IB-62 and the reference strain GM-7, respectively, in bioassays against *T. ni*, while against *H. virescens* the LC_{50} values were 184, 244 and 338 ng/cm^2 for GM-IB-7, GM-IB-62 and GM-7, respectively, while against *S. frugiperda*, the values were 472, 494 and 339 ng/cm^2 for GM-IB-7, GM-IB-62 and GM-7 respectively.

Poster – Microbial Control Monday 16:30 **MC-20**

Evaluation of native strains of *Isaria fumosorosea* (Wize) against *Anastrepha ludens* (Loew) (Diptera: Tephritidae) in Mexico

Fátima Lizeth Gandarilla-Pacheco, Héctor Daniel Nava-González, Katiushka Arévalo-Niño, María Guadalupe Maldonado Blanco, and Isela Quintero-Zapata

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The Mexican fruit fly *Anastrepha ludens* (Loew) is a pest that seriously affect fruit production in Mexico and other Neotropical countries. In Mexico, *A. ludens* is distributed mainly in the production areas of mango, orange, guava, apple and peach, covering 49.75% of the country and is able to cause about 30% damage in the production, marketing and hinder due to the imposition of strict quarantine barriers. Presently, pest control is mainly based on the use of bait insecticides to control adults and soil application of insecticides to kill larvae and newly emerged adults, but because this type of pollution control and high mortality of beneficial insects. In this study we evaluate different isolates of *I. fumosorosea* with potential for larvae and/or pupae control of *A. ludens*. There were evaluated 6 isolates of *I. fumosorosea* (HIB-27, HIB-28, HIB-29, HIB-30, HIB-32, Pfr-612) on larvae and pupae. Treatments were incubated during 72 hours at 25±2^{o}C ,60-65% R.H. and 12:12 h L: D. The results showed that HIB-27, HIB-32 caused 46% mortality while Pfr-612 showed 57% in larvae of *A. ludens*; meanwhile, in the controls (untreated and treated with 0.01% Tween 80) the mortality was 0%. In pupae of *A. ludens*, the isolates Pfr-612 and HIB-27 produced 47 and 62% mortality, respectively (0%mortality in the controls). However in both stages was presented an interesting phenomenon. Strains HIB-28, HIB-32 and Pfr-612 inhibited the process of metamorphosis by up to 58% in larvae whereas in pupae only HIB-27 achievement strain inhibits this process by 51%.

Poster – Microbial Control Monday 16:30 **MC-21**

IMBICONT: Improved biological control for IPM in fruits and berries

Italo Delalibera Jr.[1], Jørgen Eilenberg[2], Annette Bruun Jensen[2], Celeste D'Alessandro[1], Lene Sigsgaard[2], Sílvia Helena Galvão de Miranda[3]

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IMBICONT is a three year project, initiated in 2012, to strengthen bilateral scientific collaboration between scientists in São Paulo, Brazil, and Denmark, jointly funded by The State of São Paulo Research Foundation (FAPESP) and the Danish Council for Strategic Research (DSF). The aim is to provide future solutions for sustainable Integrated Pest Management in strawberries, apple and citrus. The target biocontrol organisms chosen include predators and insect pathogenic fungi. Three work packages (WP) are included: WP1 aims to explore new biodiversity of entomopathogens and co-evolution between crop, host and pathogen. Also we will perform studies on selection of new strains, new production methods, and formulation and application techniques for unexplored entomopathogenic fungi. WP2 aims to design new strategies to enhance beneficial interactions between crops, pests and their natural enemies, employing principles of the conservation biological control strategy as part of IPM. Finally, WP3 aims to integrate biological results and economic analyses into IPM in fruits and berries. A micro-economic assessment of the value of biological control will be carried out to give future directions of biological control and IPM and compare with conventional methods of pests control, as well as to evaluate the economic feasibility of its application in both countries.

Poster – Microbial Control Monday 16:30 **MC-22**

Increasing food availability by reducing crop losses for smallholder farmers

Theresa Corless, Rob Reeder and Steve Edgington

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CABI is leading a programme which aims to establish national plant health systems that link farmers with extensionists, input suppliers, plant and insect pathology labs, universities, research and government. This programme, called Plantwise, helps developing countries establish community-based plant health clinics which provide the entry point for this national system and are a means by which farmers can gain access to professional advice on plant health problems. Assessment has shown that almost all farmers tried out the treatments

recommended by the clinics, and the results were good: increased yield and crop quality, and reduced spending on unnecessary pesticides, leading to the production of big returns. In Bolivia adopters of the plant clinic advice reported an average boost to income of almost US$700 per hectare in potato farming in consecutive growing seasons (before and after adopting advice) and even more for fruits and vegetables. Most parts of the plant health system already exist within countries, but often in developing countries the parts operate independently. The plant clinics act as the lynch-pin to bring them together in an integrated system that is able to directly assist farmers.

Poster – Microbial Control Monday 16:30 **MC-23**

Improvement of the economic feasibility of baculovirus production processes in insect cell cultures by use of the effluent for the production of high-value added goods: application to the production of *Bacillus thuringiensis*

Gabriela Analía Micheloud[1,2], Verónica Viviana Gioria[1,2], Gustavo Pérez[3] and Juan Daniel Claus[1,2]

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The processes for the production of insecticidal occlusion bodies (OBs) of baculoviruses in insect cell cultures at industrial scale has been impaired by biological restrictions, that limits the yield of the viral product, as well as by economical drawbacks. Recent developments in the design of low cost media, as well as the optimization of infection conditions, driving to improved viral yields, are steps aimed at developing viable processes, but still insufficient to reach the economical feasibility. The use of effluents to produce high-value added products is a valuable strategy to improve the profitability of biotechnological processes. The effluent of a production process of insecticidal OBs in insect cell cultures is a spent medium that, because the richness of remaining nutrients, could allow the multiplication of useful microorganisms. The aim of this work was to study the feasibility to use the spent medium of UFL-AG-286 cell cultures in the low-cost UNL-10 medium, after infection with *Anticarsia gemmatalis multiple nucleopolyhedrovirus*, to sustain the growth and sporulation of *Bacillus thuringiensis var. kurstakii* (Btk) in shaken flasks. The yield of Btk spores in the viral effluent was 9.92 x 10^{11} spores per liter. This yield was 81.6% higher than that reached in control cultures made in a medium commonly used to support Btk proliferation. These results demonstrate the feasibility to use the effluent of a baculovirus production process in insect cell cultures to produce Btk, and could be a step towards a viable alternative for producing viral and bacterial bio-insecticides in an integrated process

POSTER SESSION 1 Monday, 16:30 – 18:30

VIRUSES

Poster – Viruses Monday 16:30 **V-01**

Complete sequence and genomic analysis of the *Hyphantria cunea* granulovirus

Jae Bang Choi, Won Il Heo, Sung Min Bae, Tae Young Shin, Jun Beom Lee, Yeon Ho Je[1], Byung Rae Jin[2] and Soo Dong Woo*

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A fall webworm, *Hyphantria cunea,* is considered an agricultural pest and a major pest of many board-leaved trees. Recently, we isolated *H. cunea* granulovirus (HcGV) from naturally infected larvae in Korea. To better understand HcGV, the nucleotide sequences of the DNA genome were determined, analyzed and compared to those of other baculoviruses. The HcGV genome consisted of 114,557 bp and had on overall G+C content of 39.30%. Computer- assisted analysis predicted 130 open reading frames (ORFs) of 50 amino acids or greater that showed minimal overlap. HcGV shares more than 106 ORFs with other baculoviruses. The positions of at least some baculovirus homologous repeat regions appear to be conserved relative to specific baculovirus genes. The gene parity plot analysis, percent identity of the gene homologues and a phylogenetic analysis suggested that HcGV is beta-baculovirus that is closely related to *Xestia c-nigrum* granulovirus but with a highly distinct genomic organization.

Poster – Viruses Monday 16:30 **V-02**

Occurrence and genetic variability of CpGV infecting *Cydia pomonella* at different geographical locations in Argentina

Joel D. Arneodo[1], O. Marcelo Farinon[1], Ricardo Salvador[1], Karolin Eberle[2], Alicia Sciocco-Cap[1], Johannes Jehle[2] and Graciela Quintana[1]

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Cydia pomonella granulovirus (CpGV) is world-wide used for the control of *C. pomonella*, a lepidopteran pest affecting apple, pear and walnut orchards. In Argentina, CpGV-M based biopesticides have been employed in the field since the 1990s. As a part of a broader program for preventing host resistance to the virus, a survey was conducted at different apple, pear and walnut producing areas of the country in order to achieve a better understanding of the population dynamics of this entomopathogen. Third to 5th instar *C. pomonella* larvae were sampled in regions where the viral biopesticide has been extensively used and then discontinued, and in regions with occasional or no CpGV-based product application. Samples were analyzed through PCR with CpGV-specific primers. Preliminary results showed that CpGV could be detected in larvae collected in orchards with a long history of CpGV-based insecticides even several years after the end of the applications and in untreated orchards in the surrounding areas. However, until now no virus was detected in regions where a few sporadic treatments were performed and in geographical areas away from the application sites. With regard to the genetic variability, based on the sequencing of *lef*-8 and granulin genes, no distinct virus strains were recorded so far. Further samplings will provide a more comprehensive overview on this and other issues concerning the use of CpGV in integrated pest management programs.

Poster – Viruses Monday 16:30 **V-03**

Occurrence and phylogenetic characterization of a baculovirus isolated from *Culex quinquefasciatus* in São Paulo State, Brazil.

Carlos José Pereira da Cunha de Araujo-Coutinho[1], Rafael Alves[1], Neil D. Sanscrainte[3], Andréa de Barros Pinto Viviani[2], Paulo Frugoli dos Santos[2], Polyana A. Vasconcelos-Medeiros de Souza[1], Isabel Maria Vicente Guedes de Carvalho-Mello[1]* and James J. Becnel[3]

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Baculoviruses are microbial agents that affects mosquito and lepidoptera larvae. They are characterized by rod-shaped virions containing circular double-stranded DNA and are the most studied insect viruses, due to their role as biological pesticides. The aim of this study was to assess the occurrence of viral infections in mosquitoes and characterize them by using molecular tools. Fortnightly collections were made of mosquito larvae in the city of Caraguatatuba. Six larvae of *Culex quinquefasciatus* were isolated that had white cysts (nodules) in

epithelia cells of the posterior midgut indicative of infection by a baculovirus. These larvae were subjected to DNA extraction. DNA was amplified producing a fragment of around 600nt of the lef-8 gene and 400 nt of Pif-2 gene. The sequences were aligned by ClustalX 2.0 with partial sequences of the lef-8 gene of baculoviruses isolated from other insect orders taken from genebank and edited and the phylogenetic analysis was performed. The phylogenetic analysis performed with the lef-8 and pif-2 gene demonstrated that the baculovirus identified in *Culex quinquefasciatus* in Caraguatatuba region is most closely related to that of *Culex nigripalpus* nucleopolyhedrosis baculovirus.

Poster – Viruses Monday 16:30 **V-04**

Genetic diversity among isolates of *Autographa californica multiple nucleopolyhedrovirus*

Robert L. Harrison[1], Holly J. R. Popham[2], Jonathan E. Breitenbach[2] and Daniel L. Rowley[1]

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Our knowledge of genetic variation at the nucleotide sequence level of *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV; *Baculoviridae*: *Alphabaculovirus*) derives from complete genome sequences of the C6 clonal isolate of AcMNPV and the R1 and CL3 clonal isolates of AcMNPV variants *Rachiplusia ou* MNPV (RoMNPV-R1) and *Plutella xylostella* MNPV (PlxyMNPV-CL3), along with individual gene sequences from other AcMNPV isolates. To obtain a more comprehensive view of genetic diversity among AcMNPV and AcMNPV-like viruses, sequence data of 74 virus samples from *A. californica*, *Autographa gamma*, *Trichoplusia ni*, *Rachiplusia ou*, *Anagrapha falcifera*, *Galleria mellonella*, and *Heliothis virescens* were generated by PCR. Nucleotide sequence analysis indicated that 45 samples contained isolates of AcMNPV, while six samples contained isolates of RoMNPV and 25 samples contained isolates of *Trichoplusia ni single nucleopolyhedrovirus* (TnSNPV). BLAST queries and phylogenetic inference from partial sequences of *lef-8*, *lef-9*, *polh*, *ie-2*, and the ORF *ac86* region indicated a distinct group of AcMNPV isolates characterized by an unusual ie-2 gene previously found in PlxyMNPV-CL3 and a large deletion within *ac86* originally described in the AcMNPV isolate 1.2. PCR and sequence analysis of *bro* genes suggested that the *bro* gene *ac2* had been formed by a deletion that fused two adjacent *bro* genes together. In bioassays against *T. ni* larvae, significant differences were observed in the insecticidal properties of individual isolates, but no trends were observed among the AcMNPV, TnSNPV, or RoMNPV groups of isolates. This study expands on what we know about genetic diversity within AcMNPV and AcMNPV-like viruses.

Poster – Viruses Monday 16:30 **V-05**

Complete sequence comparison between three genetically distinct *Bombyx mori* nucleopolyhedrovirus isolates in Korea

Won Il Heo, Jae Bang Choi, Sung Min Bae, Tae Young Shin, Jun Beom Lee, Yeon Ho Je[1], Byung Rae Jin[2] and Soo Dong Woo*

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The genome of three genetically distinct isolates of *Bombyx mori* nucleopolyhedroviruses (BmNPVs), BmNPV-K1, K4 and K5 strains isolated in Korea was completely sequenced and comparative analyzed. BmNPV-K1 consisted of 126,747 bp and 131 open reading frames (ORFs) of 150 nucleotides or longer with minimal overlap have been identified. In contrast, BmNPV-K4 and K5 consisted of 128,618 bp and 134 open reading frames (ORFs), 127,554 bp and 133 open reading frames (ORFs), respectively. Although gene arrangement is virtually identical, the genome of BmNPV-K4 was 1,871 bp and 1,064 longer than that of BmNPV-K1 and K5, respectively. The most interesting difference between these viruses was the presence or absence of baculovirus repeated ORFs (bro) genes. To investigate the relationship between BmNPV-K1, K4 and K5, phylogenetic analysis with each member of the paired ORFs was performed. The sequence data suggest that BmNPV-K1, K4 and K5 are closely related but have diverged and evolved into three separate strains. This was study to identify highly related but separately evolving viruses in the same insect host and geographic location. We are currently comparing the differences of these BmNPV genomes to elucidate function of novel genes.

Poster – Viruses Monday 16:30 **V-06**

Lack of stability of the infectivity of budded virus of *Anticarsia gemmatalis multiple nucleopolyhedrovirus* in serum-free medium supplemented with lipid microemulsions

Ignacio Eberhardt[1,2], Verónica Viviana Gioria[1,2], Gabriela Analía Micheloud[1,2] and Juan Daniel Claus[1,2]

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Biotechnological applications of baculoviruses are constantly expanding, from bioinsecticides to protein expression and gene therapy vectors. The development of feasible processes for the production of baculovirus and baculovirus-based products in insect cell cultures requires that high-titer stocks of budded virus (BVs) being prepared and stored in serum-free medium. It is known that the preservation of baculovirus BVs by freezing at ultra-low temperatures in serum-free medium is less efficient than in culture media added with serum, but the causes were not elucidated. The aim of this work was to study the stability of the infectivity of BVs of the *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV) in the UNL-10 serum-free medium under different conditions of supplementation with lipid microemulsions, freezing at -80°C, and time of exposure to 27°C, employing a 2^3 full factorial design in duplicate. The exposure to 27°C, as well as the freezing and thawing of an AgMNPV stock, did not affect the stability of the viral titer determined through an end-point dilution method. On the other hand, the exposure to lipid microemulsion provoked a significant negative effect on the stock titer. This deleterious effect was magnified when BV samples in medium supplemented with lipid microemulsion were frozen and thawed, losing more than 90% of the viral infectivity. These results strongly suggest that the reduced stability of AgMNPV BVs in serum-free media is associated to the presence of lipid microemulsions, and indicate the necessity to establish alternative protocols to store BVs stocks produced in serum-free medium supplemented with lipid microemulsions.

Poster – Viruses Monday 16:30 **V-07**

Sequential passage of the Nucleopolyhedrovirus of *Anagrapha falcifera* (AfMNPV) in larvae of *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae)

Fabiane Cunha[1,2]; Flavio Moscardi[1,2]; Maria E.B. Castro[3], Moema T. Castro[3], Zilda M.A. Ribeiro[3], Ângela Falleiros[1], Sheila M. Levy[1], Mauricio L. Moscardi[1], Talita M. Alexandre[1] and Daniel R.Sosa-Gomez[4]

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Among the insect pests of cultivated plants in Brazil species of the *Spodoptera* genus are very important, causing economic damage to different crops such as soybean, corn and cotton. This work had the objective of evaluating *S. cosmioides* larval mortality after sequential passage of an isolate of the AfMNPV through generations F1 to F6. Two hundred second-instar larvae were used in each passage and larvae were evaluated up to the eleventh day after viral inoculation (DAI). Dead larvae collected after each passage were macerated and centrifuged at 10,000 rpm for 15 minutes for continuity of the subsequent passage and viral concentration was standardized at $1x10^8$ OB/ml. Mortality of *S. Cosmioides* due to the AfMNPV increased from 2.38% in F1 to 84.5% in the F6 passage. Also, peak mortality occurred on the 6th DAÍ in F6 as opposed to peak mortality on the 7th DAÍ in F3, F4, and F5 We also extracted and purified the DNA from OBs obtained in F2 e F10 and for DNA clivage we used the restriction enzymes ECORI, HINDII, PstI. The DNA fragments were run through agarose gel electrophoresis and the protein profiles were different for F2 and F10. We also observed some differences among the restriction profiles of the viral isolates, mainly for the digestion with the ECORI enzyme that revealed a larger spectrum of variation regarding number of detectable DNA fragments.The results indicate that the AfMNPV although not specific to *S.cosmioides* can be virulent to this species, what demand further studies regarding its use pest management programs in crops where the insect is important.

Poster – Viruses Monday 16:30 **V-08 STU**

Analysis of recombinant protein expression in *Anticarsia gemmatalis* larvae infected with recombinant AgMNPV baculoviruses containing the firefly luciferase gene under the control of early and late promoters

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The *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV) is one of the best examples of the use of a virus to control an insect pest in agriculture worldwide. This baculovirus infects larvae of *Anticarsia gemmatalis* moth (Lepidoptera), a soy bean pest, and has been used as a successful biocontrol agent in Brazil for over two decades. This depends on the unique ability to generate two viral phenotypes, one responsible for the viral survival in the environment outside the host and the agent of primary infection, which consists of viral particles encapsulated into a polyhedral shaped protein crystal, the occlusion body (OB), which is used as a bioinsecticide, and a second phenotype, a budded virus (BV), which is a single membrane-containing viral particle. To follow the progress of infection in terms of the accumulation of viral proteins inside the host, we infected fourth instar *Anticarsia gemmatalis* larvae by intrahemocoelic injection, with recombinant AgMNPV expressing the Firefly Luciferase (FLUC) gene, driven by the *ie1, gp64, vp39, p6.9* and *polh* promoters, we then quantified the luminescence in the hemolymph using a luminometer and visualized the progress at different times post-infection by injecting luciferin into the larvae, which glowed at different times depending on the recombinant virus used. The expression of luciferase driven by the *p6.9* and *vp39* promoters showed a quick accumulation of protein, while late in infection there is hyperexpression from *polh* promoter. The results show new options in the design of vectors based on AgMNPV for recombinant protein expression in insect larvae.

Poster – Viruses Monday 16:30 **V-09**

Prediction and detection of a viral microRNA in AgMNPV infected High Five cells

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MicroRNAs (miRNAs) have emerged as key players in host–pathogen interactions. Although many virus encoded miRNAs have been identified in different mammalian species, this type of molecules has hardly been investigated to elucidate their role in baculovirus-insect host interaction. A recent study revealed that baculovirus encoded miRNAs were evolutionarily conserved among some closely related members of the *Alphabaculovirus* genus, group I. In this study, we started an *in silico* search for miRNA genes encoded by the *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (AgMNPV) genomic DNA based on the comparison with the previously identified *Bombyx mori* nucleopolyhedrovirus (BmNPV) miRNAs. The late gene *lef-8* was reported as a putative *bmnpv-mir-4* target. Complementary *miR-4* seed region was fully conserved in AgMNPV and BmNPV even though identity percentage in the gene sequence is around 75%. Sinteny analysis allowed us to select regions to look for the *miR-4* coding gene in AgMNPV through seed region searching and a number of putative *agmnpv-miR-4* sequences were found. In order to find the pre-miRNA, flanking sequences (~100 nt) of putative mature *agmnpv-miR-4* were analyzed using Mfold program for secondary structure prediction. A single pre-*miR-4* structure was selected with a free energy change (ΔG) of −25.6 kcal/mole as the candidate precursor. Northern blot hybridization was performed on samples from both AgMNPV infected and uninfected Hi5 cells using an end-labeled oligonucleotide complementary to the predicted *agmnpv-miR-4* as probe. In a preliminary assay, specific signal was detected in RNA samples isolated on day 6 post infection. Further studies aiming to find new AgMNPV encoded miRNAs involved in the regulation of other crucial targets will allow us to understand the role of these miRNAs in virus-host interaction.

Poster – Viruses Monday 16:30 **V-10 STU**

Proteomics of the *Anticarsia gemmatalis* multiple nucleopolyhedrovirus budded viruses

Diego Luis Mengual Gómez, Mariano Nicolás Belaich and Pablo Daniel Ghiringhelli

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Baculoviridae is a viral Family that infects different insects, most of them plagues for numerous agriculture crops. Consequently, their characterization is important for the development and production of new and better bioinsecticides. The baculoviruses produce two phenotypes along its viral cycle. The Occluded Derived Viruses (ODV) are responsible for the primary infection in the insect midgut cells, while the other phenotype, the Budded Viruses (BV), are responsible for systemic infection. To study the possible factors that state the host range has been studied the protein composition of several ODVs. However, there is only one proteomic analysis reported for BVs. Seeking to enrich the knowledge about this phenotype, this work were conducted to determine the BV structural proteome of AgMNPV. The analysis was carried out using the technology of two-dimensional gel electrophoresis and subsequent identification by mass spectrometry. AgMNPV was multiplied in UFL-Ag-286 cells and the BVs were isolated from the culture medium. Then, their integrity and purity was checked by transmission electron microscopy. Once obtained the sample the protein extractions were performed, which were resolved by two-dimensional gel electrophoresis. This technique allowed detecting about 100 different polypeptides, which were identified by mass spectrometry MALDI-TOF-TOF.

Poster – Viruses Monday 16:30 **V-11 STU**

Evaluation of AgMNPV replication based on HRs sequences

Solange Ana Belen Miele, Mariano Nicolas Belaich and Pablo Daniel Ghiringhelli

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The baculovirus *Anticarsia gemmatalis* MNPV (AgMNPV) is the most extensively used virus pesticide in the world to control the velvetbean caterpillar, *A. gemmatalis*. Baculoviruses have been used in many other biological applications such as protein expression systems, models of genetic regulatory networks and genome evolution, and putative non-human viral vectors for gene delivery. These viruses are arthropod-specific viruses containing large double-stranded circular DNA genomes of 80,000–180,000 bp. The progeny generation is biphasic, with two different phenotypes during virus infection: budded viruses (BVs), during the initial stage of the multiplication cycle, and occlusion-derived viruses (ODVs), at the final stages of replication. It seems that baculoviruses have evolved multiple regions that can function as origins of replication. Most baculoviruses contain AT-rich homologous regions (hrs) interspersed around the genome. The number and distribution of hrs is variable in different species of baculovirus. It has been postulated that hrs function as viral origins of replication because of their symmetric location, high AT content, and palindromic structure. Other AT-rich regions but non containing hrs (Non hr-Ori) and several early promoter regions have been characterized also as potential origins of replication in transient assays. In this work, the replication of a series of plasmids, each containing one or more hrs from AgMNPV was investigated in infected UFL-Ag286 cells. To obtain those plasmids, AgMNPV genome were segmented in 8 fragments that were cloned in pZErO-2 and *Escherichia coli* DH10B. The levels of plasmid multiplication in infected cells were quantified and compared among them.

Poster – Viruses Monday 16:30 **V-12**

Susceptibility evaluation of six insect cell lines to *Spodoptera frugiperda multiple nucleopolyhedrovirus*

Jorge O. Mateus[1], William Sihler[1], Zilda Maria A. Ribeiro[1], Fernando H. Valicente[2] and Marlinda L. Souza[1]

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Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) is a baculovirus pathogenic to the fall armyworm, an important polyphagous pest in South America. Due to current limitations to its *in vivo* production and in order to develop studies on cell culture viral production, in the present work the susceptibility of six insect cell lines to the SfMNPV was analyzed. Initially, cells were incubated with SfMNPV (I-19 isolate) Budded Virus, for 1h adsorption time, and kept in TNMFH medium with 10% FBS at 27^{0}C, for seven days. The cytopathic effects induzed by the virus were then monitored by phase contrast microscopy. In addition, the kinetic of the viral protein synthesis was carried out using 35S-methionine labeling followed by SDS-PAGE and autoradiography. Morphological analysis showed that the two cell lines of *Spodoptera frugiperda* (IPLB-SF-21AE and Sf9) were very productive with typical alterations caused by baculovirus infection leading to many polyhedra formation in the cell nucleus. Besides, *Lymantria dispar* cells (IPLB-LD-625Y) became highly vacuolated while *Bombyx mori* cells (BM-5) changed its shape from round refractive to "groundnut", although none of them had polyhedra production. On the other hand, *Anticarsia gemmatalis* (UFL-AG-286) and *Trichoplusia ni* (BTI-Tn-5B1-4) showed no visual morphological alterations. As expected, viral proteins kinects by radioactive pulse labeling revealed mainly a strong polyhedrin synthesis in IPLB-Sf21 and Sf9 cells after 48hpi. The remaining cell lines showed a similar protein profile to the control cells, despite the fact that IPLB-LD-625Y and BM-5 cells presented unusual morphological signs of infection.

Poster – Viruses Monday 16:30 **V-13**

Ultrastructural analysis of six *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV) Many Polyhedra variants

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Baculovirus has been extensively used for agricultural and forest pest control. So far, commercial production has been made by viral growth in the host insect (*in vivo*). On the other hand, *in vitro* production presents strong limitations due to generation of mutants during serial passage of the virus in cell culture. An important strategy to optimize the baculovirus *in vitro* production is the selection of Many Polyhedra (MP) variants. These variants are more stable and form many polyhedra in the cell nucleus even after consecutive passages in cell culture. This work presents the ultrastructural analysis of six Many Polyhedra variants of *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV). These variants were selected by plaque assay after seven serial passages of the prototype AgMNPV-2D in Tn5B1-4 (High Five™) cells. Determination of their specific occlusion body (OB) production showed an average of 200 OB/cell. In order to visualize the cytopathic effects, High Five cells were infected with each MP variant, transferred to a fixer solution at 72h p.i. and treated for electron microscopy. The samples were then stained with 3% uranila acetate and photographed using a Jeol 1011 transmission electron microscope. The cells showed the same baculovirus typical effects infection such as cell rounding, nuclear hypertrophy, virogenic stroma formation, virus assembly and polyhedra production. No morphological differences were observed among the six AgMNPV Many Polyhedra variants and the parental virus (AgMNPV-2D). In following studies, the size of the Many Polyhedra variants polyhedra will be determined using a scanning electron microscope.

Poster – Viruses Monday 16:30 **V-14**

Laboratory and field populations of *Spodoptera exigua* are naturally infected by multiple viruses

Cristina Virto[1], David Navarro[1,3], Mª del Mar Tellez[3], Salvador Herrero[4], Trevor Williams[5], Rosa Murillo[1,2], Primitivo Caballero[1,2]

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Covert infections of *Spodoptera exigua* multiple nucleopoliedrovirus (SeMNPV) have been detected in laboratory or field populations of the homologous host, *S. exigua*. Recently, RNA viruses belonging to the *Iflaviridae* family were identified in *S. exigua* transcriptome from different laboratory colonies (*S. exigua iflavirus*-1: SeIV-1; *S. exigua iflavirus*-2: SeIV-2). The three viruses are vertically transmited and establish persistent infections, and coinfection of individual insects by these viruses is considered likely. Very little is known about iflaviruses but they have been reported in association with NPVs in previous studies. In this study, we determined the prevalence of covert infections caused by iflaviruses and SeMNPV in order to identify virus associations in natural *S. exigua* populations. Field adults were captured with UV light-

traps and their offspring reared in laboratory conditions. RT-PCR and qPCR were used to detect iflaviruses and SeMNPV, respectively. SeMNPV was detected in the 20% of field-adults, whereas 15% and 5% of insects were infected by SeIV-1 and SeIV-2, respectively. The adults of F_1 are now being analyzed to determine the prevalence of each of the viruses. A laboratory colony used as reference, showed 74% (n=39) and 84% (n=19) of adults were infected by SeIV-1 and SeIV-2; non-SeMNPV amplifications were detected by qPCR (n=20).

Poster – Viruses Monday 16:30 **V-15 STU**

The role of *Spodoptera exigua* multiple nucleopolyhedrovirus genes *se76* and *se28* on viral pathogenicity

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Six Spanish genotypes of the *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) differ in their efficiency for vertical and horizontal transmission as well as in their pathogenicity (mean lethal dose), virulence (speed of kill) and productivity (number of progeny virus per insect). Whole genome sequencing of these genotypes could pinpoint genes involved in host-pathogen interactions. The slowest and fastest killing viruses had a mutation in the SeMNPV ORFs *se76* (*cg30*) and *se28*, respectively. The *cg30* encoded protein, featuring two nucleic acid-binding sites, a zinc finger, and a leucine zipper, is considered a prime candidate for the regulation of baculovirus late expression products. The function of *se28* is not known, although it has been determined as non-essential because of the existence of natural viral genotypes lacking this gene that can replicate both *in vitro* and *in vivo*. In this study we examined the influence of these genes in the pathogenicity of SeMNPV with two knockout recombinants, SeBAcAL1null76 and SeBacAL1null28, which were constructed using the bacmid-based recombination system.

Poster – Viruses Monday 16:30 **V-16**

Sex-specific variation in vertical transmission of SeMNPV

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Vertical transmission of baculovirus naturally occurs in lepidopteran populations, and has been proposed as a strategy for virus survival during periods of host scarcity or for improving virus dispersal. Previous studies established that the nucleopolyhedrovirus of *Spodoptera exigua* (SeMNPV) is vertically transmitted in field-caught adult moths from Almerian (Spain). Unexpectedly, field-caught gravid females were observed to produce virus-infected offspring even though no evidence of the infection was seen in these females. This led us to suspect that both sexes may contribute to vertical transmission of the pathogen. To examine this hypothesis we set up a mating schedule involving four groups: i) virus-free males × virus-free females; ii) infected males× infected females; iii) virus-free males × infected females; and iv) infected males × virus-free females. The offspring from each treatment were individually reared through to adults and then analyzed by qPCR. We will present the results of this experiment and show gender-specific differences in the capacity to transmit SeMNPV to the offspring.

Poster – Viruses Monday 16:30 **V-17**

Biological comparison of four nucleopolyhedrovirus isolates of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)

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Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae), originally from southeastern Asia, is now an important cosmopolitan pest of vegetable crops. Larvae of this species are susceptible to a nucleopolyhedrovirus (NPV), which has attracted interest as a potential biocontrol agent. Four strains of NPV isolated from infected *S. exigua* larvae in Mexico were subjected to biological comparison in terms of infectivity (median lethal concentration, LC_{50}) and virulence (median survival time, MST). A NPV isolate from Florida (SpoD-X®) was also included in the bioassays. Droplet feeding bioassays with 2nd instars indicated that the CL_{50}s values calculated for the isolates from San Luis Potosí state collected in 2006 (SLP-2006, 1.07 x 10^6 OBs/ml) and 2008 (SLP-2008, 3.92 x 10^6 OBs/ml), were significantly higher than the value calculated for Spod-X® (3.82 x 10^4 OBs/ml), according to the confidence limits at 95%. The CL_{50} value for isolate collected from Sinaloa state in 2006 (SIN-2006, 9.75 x 10^4 OBs/ml) was not statistically different to Spod-X®, although CL_{50} value calculate for isolate collected in Sinaloa state in 2008 (SIN-2008, 4.11 x 10^3 OBs/ml) was significantly lower compared with the latter. No significant differences were detected in the MST among any isolates [SpoD-X® (121 h), SIN-2006 (122 h), SIN-2008 (125 h), SNLP-2006 (120 h), and SNLP-2008 (117 h)]. Currently, genetic comparisons of all isolates are under study by using polymerase chain reaction (PCR)-amplification and sequencing of different genes and homologous regions (hr).

Poster – Viruses Monday 16:30 **V-18**

Combined effects of azadirachtin and a nucleopolyhedrovirus (SfMNPV) on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae

Norma Zamora Avilés[1] Jorge Alonso Vargas-Leandro[2], Samuel Pineda-Guillermo[1], José Isaac Figueroa de la Rosa[1], Juan Manuel Chavarrieta[1] and Ana Mabel Martínez-Castillo[1]

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Azadirachtin (AZA), a tetranortriterpenoid compound derived from the neem tree, *Azadirachta indica* A. Juss (Meliaceae), has insecticidal activity against phytophagous Insects. Diet surface contamination bioassays were performed with *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) and AZA alone and in mixtures on 3th instar of *S. frugiperda*. The combined treatment of SfMNPV (370 OBs/mm^2) with AZA resulted in higher percentage of mortality than the virus alone treatment in one concentration (5.3 mg a.i./L^{-1}) at 168 and 192 h after treatment and two concentrations (5.3 and 10.5 mg a.i./L^{-1}) at 216 h after treatment. To determine the effect of SfMNPV-AZA mixtures on the development of *S. frugiperda* and on the virus production, a bioassay was performed using a mixture of SfMNPV (430 OBs/mm^2) and AZA (26.4 mg i.a) in a water solution. The total duration of larval development (3th and 4th instar) increased significantly in larvae exposed to SfMNPV-AZA mixtures and AZA alone compared to the SfMNPV alone and the control. The total yield of OBs per larvae and OBs per mg larval weight in SfMNPV alone was 7.9 and 1.8-fold higher, respectively, than the yields from insects inoculated with the SfMNPV-AZA mixtures, consequently AZA should not

be considered for baculovirus *in vivo* mass production. Despite the positive aspects that favor AZA + NPV mixtures, studies on field efficacy under commercial growing conditions also are required to demonstrate that the potentiation observed in the laboratory translates to the field.

Poster – Viruses Monday 16:30 **V-19 STU**

Feeding, growth and toxicity evaluation of microbial insecticides *Spodoptera* Nucleopolyhedrovirus (splt NPV) against *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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The Microbial insecticides of *Spodoptera* Nucleopolyhedrovirus (splt NPV) is insect-specific baculoviruses provide to control of serious pest of *Spodoptera litura* (Tobacco cutworm) were evaluated in the laboratory. In the present study carried out the various concentration of splt NPV (10^2,10^4 and 10^8) was evident Growth, fecundity, food utilization and mortality of *S. litura.* Life history parameters after the treatment showed considerable effect and extended larval (17.1 to 29.9 days,) and pupal duration (9.5 to 14.2 days) decreased male and female longevity (Male 7.4 to 3.6 days and Female 8.7 to 4.1 days) and fecundity (1750 to 882 Nos.). Nutritional efficiency measures were significantly decreased after the treatment. The Nutritional indices were decreased CI (0.0948 to 0.0885 g), RGR (0.0188 to 0.0156 g), ECI (19.78 to 17.64 %) and ECD (22.78 to 17.74 %) and increased AD (86.80 to 89.41 %) in IV instars of *S. litura* larvae. Mortality of larvae was also evident and lethal concentrations LC_{50} and LC_{90} values were also presented. 100% mortality was evident in I instar larvae of *S. litura*. The value of LC_{50} was 2995.69 % and LC_{90} was 17643.51 %, respectively.

TUESDAY 7th

Symposium II Monday, 08:00-12:00

Microsporidia Division

Microsporidia from South America

Symposium II Tuesday, 8:00 **35**

***Edhazardia aedis*, a microsporidian pathogen of *Aedes aegypti*: Possibilities and challenges for classical biocontrol in South America**

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Edhazardia aedis, a pathogen of *Aedes aegypti,* has a complex life cycle involving both horizontal and vertical transmission affecting two successive generations of the host. Usually, one sporulation sequence occurs in the adult female (infected orally as a larva) and results in the formation of binucleate spores. These spores are involved in vertical transmission of *E. aedis* to the subsequent generation via infected eggs. In infected progeny, larval death results in the release of uninucleate spores that are responsible for horizontal transmission when ingested by larvae. This developmental sequence leads to the formation of binucleate spores in the adult to complete the cycle. Optimism regarding the role of *E. aedis* as part of a program to control *Ae. aegypti* focuses on a number of desirable traits. Both the vertical and horizontal components of the life cycle of *E. aedis* are highly efficient providing the means for the parasite to become established, persist and spread in populations of *Ae. aegypti*. *Edhazardia aedis* has a profound effect on the reproductive capacity of these infected adults with a 98% reduction in overall fitness by reducing survival, fecundity and the percent of eggs that hatch. Good persistence is expected in release sites due to life cycle flexibility with dissemination to other mosquito-inhabiting sites by means of vertical transmission. Survival during dry periods occurs within the mosquito eggs where the parasite can survive for the life of the egg. The possibilities and challenges of utilizing *E. aedis* as a classical biocontrol agent will be discussed.

Symposium II Tuesday, 8:30 **36**

Native and alien microsporidia in Argentine grasshoppers

Carlos E. Lange

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Cooperative projects between scientists of the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) and La Plata National University (UNLP) in the late 1970´s and early 1980´s initiated the study of microsporidia as potential biocontrol agents of grasshoppers in Argentina, a previously unexplored field of research in the country. As elsewhere in the world, absolute reliance on chemical insecticides against some of the 10-15 pest grasshopper species of the country provided the justification underlying the search for more environmental-friendly alternatives of control. Since then, three native microsporidia have been described (*Liebermannia dichroplusae, Liebermannia patagonica, Liebermannia covasacrae*), several other *Liebermannia*-like isolates have been detected, and the fate of the introduced microsporidium *Paranosema locustae* was monitored for years in the Pampas, and to a lesser extent in two other introduction areas in Patagonia. The presentation will review the state of knowledge on the microsporidia associated with grasshoppers in Argentina. None of the native species so far discovered appear to be useful for control purposes due to constraints in transmission, host range, and pathogenecity. On the contrary, *P. locustae*, well established in the western Pampas and in Loncopué, Neuquén province, seems to be of value as a long-term control factor. Simultaneously, some non-target grasshopper species may be negatively affected. However, in spite of the apparent usefulness of *P. locustae* against grasshoppers in Argentina, aside from some incipient interest by organic farmers, what has been called “the chemical paradigm” still prevails and no use of *P. locustae* is being done.

Symposium II Tuesday, 9:00 **37**

Microsporidian isolates from mosquitoes of Argentina

María Victoria Micieli,[1] Theodore G. Andreadis[2], Charles R. Vossbrinck[2], James J. Becnel[3] and Juan José García[1]

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Microsporidia are among the most common and widely distributed microbial pathogens associated with mosquitoes in nature. Since 1980 studies of microsporidia in mosquitoes of Argentina were conducted at the Laboratory of Insect Vectors of CEPAVE. Eleven morphologically unique species of microsporidia belonging to the genera *Amblyospora* (8), *Parathelohania* (2) and *Hazardia milleri* were isolated from species of *Anopheles*, *Culex* and *Ochlerotatus*, while eight species still remain under consideration. The complete life cycle including the phase in the adult mosquito, the larva and in the intermediate host has been elucidated in three species of *Amblyospora* and in one species of *Parathelohania.* Molecular data on the small subunit of the ribosomal gene of 5 species of *Amblyospora* were obtained to establish the affinity of these

species to other described microsporidia of mosquitoes available from GenBank. SSU rDNA sequences obtained from these 5 species of microsporidia were unique when compared with GenBank entries. Phylogenetic tree constructed by Neighbor Joining analyses yielded high degree of congruence between parasite and host at the generic level. In this analysis *A. camposi* from *Cx. renatoi* clusters with other *Amblyospora* spp. from *Culex* mosquitoes, while *A. albifasciati* (*Oc. albifasciatus*) and *A. criniferis* (Oc. *crinifer*) group with other *Amblyospora* spp. from *Aedes/Ochlerotaus* mosquitoes. The positions of 2 microsporidia from *Psorophora* mosquitoes are unresolved. This is consistent with studies with microsporidia from other parts of the world and supports the hypothesis for coevolution between the microsporidia and its host mosquito at the generic level suggesting a degree of host-parasite co-speciation.

Symposium II Tuesday, 9:30 **38**

Microsporidia from honey bees and bumble bees in southern South America.

Santiago Plischuk[1], Mariano Higes[2] and Carlos E. Lange[1]

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Knowledge on microsporidia of honey bees and bumble bees in South America was historically limited. However, in recent years surveys have been intensified in honey bees and initiated in bumble bees. Worldwide three species of genus *Nosema* are recognized as pathogens of *Apis mellifera* and *Bombus* spp. *Nosema apis* was the only reported species parasitizing *A. mellifera* in southern South America. Like in several other parts of the world, presence of this pathogen appears now diminished compared to the emergent species *Nosema ceranae*. In fact, nowadays *N. apis* is difficult to detect, at least in most of Argentina. On the other hand, *N. ceranae* have been widely detected, not only in honey bees, but also in three species of native bumble bees (*Bombus atratus*, *Bombus morio*, and *Bombus bellicosus*) under different environmental conditions. *Nosema bombi*, one of the most common pathogens of *Bombus terrestris* in the northern hemisphere, has not been detected. In addition, what could be a novel microsporidium was recently isolated from *B. atratus*. Development stages, spore appearance, and sites of infection seem to show some resemblance to *Nosema bombi*. However, a multiplex PCR using primers from *N. ceranae* (218MITOC), *N. apis* (321APIS), and *N. bombi* (BOMBICAR) did not produce positive matches.

Symposium III Tuesday, 8:00-10:00

Fungi Division

Host Immune Response to Fungal Pathogens

Symposium III Tuesday, 8:00 **39**

Metapleural gland secretion, an extra anti-fungal cuticular immune system of leaf-cutting ants

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Ants have paired metapleural glands (MG) that produce secretions for prophylactic hygiene. This organ was a key innovation that allowed ants to diversify while elaborating nesting in microbe-ridden soil. The leaf-cutting ants have particularly well developed MGs, and use the secretions to protect themselves and their fungus gardens from infections. In a set of controlled experiments with spores of five fungi, we confirmed that known insect pathogens are also virulent ant pathogens, that *Escovopsis* fungus garden disease does not affect the ants, and that saprophytic fungi are mild pathogens or weeds. We show that the ants adjust their amount of MG secretion to the virulence of the fungus they are infected with. Finally, we applied fixed volumes of MG secretion of ants challenged with constant doses of the five fungi to agar-mats of the same fungal species. This showed that inhibition halos were significantly larger for ants challenged with virulent and mild pathogens/weeds than in controls and *Escovopsis*–challenged ants. We contend that the MG defense system of leaf-cutting ants has all major characteristics of an additional cuticular immune system, with specific and non-specific components of which some are constitutive and others induced.

Symposium III Tuesday, 8:15 **40**

Avoidance of insect pathogenic fungi by predatory insects

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Fungal entomopathogens are ubiquitous within the below- and aboveground environment and susceptible insects are predicted to be under selection pressure to detect and avoid virulent isolates. Recent evidence suggests that arthropods foraging for food and oviposition sites assess their environment for the risk of predation both to themselves and to their offspring. The detection and avoidance of entomopathogens is important for host survival, longevity and ultimately fitness; infection by an entomopathogen is often lethal to the host, thus fitness is significantly reduced. Besides studies on social insects, few studies have assessed the detection and avoidance of lethal pathogens by solitary arthropods even though pathogenic bacteria, viruses and fungi are ubiquitous worldwide and common natural enemies of many species. Here we provide an overview of the studies examining the avoidance behaviour of predatory insects in response to insect pathogenic fungi from a life history perspective. We predict that such behavioural responses are widespread and driven by the high cost of fungal infection to a host.

Symposium III Tuesday, 8:30 **41**

Fungal pathogens and temperature stress affect gene expression patterns in bees

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Most of what is known about insect immunity comes from a few model species, but genomic methods now provide a means for applying this knowledge to lesser studied insects, such as bees. We identify 116 immune response in the alfalfa leafcutting bee (*Megachile rotundata*) using comparative gene sequence methods, and then evaluated expression levels of immunity genes and some abundant genes when bee larvae were exposed to a moderate environmental stress (various temperatures), and a fungal pathogen, *Ascosphaera aggregata* (chalkbrood). Many of the alfalfa leafcutting bee immunity genes were highly conserved with honey bees and other insects. Overall, temperature stress (both high and low) led to an increased expression of many these genes. When larvae were exposed to the pathogen, the incidence of disease was lower for temperature-stressed bees. Also, bees exposed to both temperature stress and the pathogen had a greater production of stress-related genes, such as ROS, than did non-infected insects. The energy and resources required to respond to stress and pathogen invasion are thought to present evolutionary trade-offs for organisms. We hypothesize that some genes play a dual role, assisting in both temperature

stress repair and immunity, thus helping insects simultaneously deal with these two potential sources of mortality.

Symposium III Tuesday, 9:00 **42**

An antifungal defense strategy in termites and woodroaches

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Termites are extremely abundant, despite living in crowded conditions and microbe-rich environments that promote the rapid spread of disease. Their success depends in part on proteins produced by the salivary gland for antifungal defense, which include termicins (defensins) and β-1,3-glucanases (Gram Negative Bacteria-binding Proteins or GNBPs). These proteins appear to have been co-opted from an internal role in the innate immune system to an external role that relies on social behaviors such as grooming, cannibalism, necrophagy and burying of corpses. Inhibition or suppression of these proteins increases the susceptibility of termites to infection by local strains of *Metarhizium anisopliae*. *Cryptocercus* woodroaches, which represent the closest living relatives to termites, also employ secreted termicins and β-1,3-glucanases for defense against fungal pathogens. This system may therefore have evolved over 150 million years ago and facilitated the evolution of group living in termites.

Symposium III Tuesday, 9:30 **43**

Sensitivity of behavior to pathogen-related odor in the termite, *Coptotermes formosanus*

Aya Yanagawa[1], Nao Fujiwara-Tsujii[2], Toshiharu Akino[3], Tsuyoshi Yoshimura[1] and Susumu Shimizu[4]

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Entomopathogen resistant behaviors were reported in many social insects. Termites remove foreign organisms, such as fungal conidia, from the body surface of their nestmates by mutual grooming behavior. As various pathogens coexist in the termite habitat, the termites would be involved in complex interactions with such pathogens. Our first step to examine these interactions was to compare the odor impact of pathogens on termite hygiene behaviors. We studied the behavioral response of the termite, *Coptotermes formosanus* to six isolates of entomopathogenic fungus, *Metarhizium anisopliae* 455*, M. anisopliae* UZ, *Beauveria brongniartii* 782, *B. bassiana* F1214, *Paecilomyces fumosoroseus* K3 *and P. fumosoroseus* 8555. The previous results suggested that termite avoid the fungal odor at the most early „encountered stage", and the odor information enhanced the grooming and attack behaviors. The behavioral changes seem to be related with odor information. In this study, we applied 4 chemical substances related with the pathogens, which were identified by GC/MS analysis. A Y tube test indicated that 3-octanone induced avoidance at the lower concentrations but they are indifference to the odor at artificial higher concentrations. Termites possess high ability to detect pathogen related odor.

Contributed Papers Tuesday 8:00-10:00

Bacteria 2

Contributed Papers Bacteria 2 Tuesday, 8:00 **44**

Susceptibility of *Aedes aegypti* populations to *Bacillus thuringiensis israelensis* with different status of organophosphate resistance

Maria Alice V. Melo-Santos, Elisama E. Helvecio, Ana Paula A. P. Araújo, Diego D. F. A. Diniz, Andréa N. Souza, Rosineide R. A. Barros, Claúdia M. F. Oliveira, Constância F. J. Ayres and Maria Helena N. L. Silva-Filha

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The utilization of *Bacillus thuringiensis israelensis* (Bti) for controlling *Aedes aegypti* larvae is likely to expand worldwide in order to overcome resistance to chemical insecticides and environmental concerns related to the use of these compounds. The major goal of this study was to evaluate the Bti susceptibility of *Ae. aegypti* populations from Brazil displaying different status of temephos resistance. Field samples composed of eggs collected in eleven Brazilian municipalities and two laboratory colonies, one resistant to temephos (RecR) and the other a susceptible reference colony (Rockefeller), all maintained under insectarium conditions, were used in this study. Third instar larvae were submitted to bioassays, according to the reference protocol in order to determine the LC_{50} and LC_{90} of Bti towards larvae. Status of temephos susceptibility and activity of detoxifying enzymes related to the resistance to chemical insecticides in these samples, were previously characterized. All *Ae. aegypti* samples tested were susceptible to Bti, since LC_{50} and LC_{90} were similar to those established for the Rockefeller reference colony. Values of LC_{50} and LC_{90} (mg/L) showed variations in the range of 0.008-0.015 and 0.025-0.043, respectively and resistance ratios found were bellow 1.9-fold. Temephos resistance ratio as high as 280-fold, exhibited by the RecR colony, as well highly altered levels of detoxifying enzyme detected in some samples, did not interfere with the pattern of Bti susceptibility found among them. Data showed the absence of cross-resistance among these larvicides and demonstrate that Bti can be a tool to manage pre-existing organophosphate resistance among populations. (Support: FIOCRUZ)

Contributed Papers Bacteria 2 Tuesday, 8:15 **45**

Novel mutations associated to *Bacillus sphaericus* resistance are identified in a polymorphic region of the *Culex quinquefasciatus cqm1* gene

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Bin toxin from *Bacillus sphaericus* acts on *Culex quinquefasciatus* larvae through binding to Cqm1 midgut-bound receptors and disruption of *cqm1* gene is the major cause behind resistance. The goal of this work was to screen for a laboratory-selected resistance $cqm1_{REC}$ allele in field populations of Recife city (Brazil) and to identify novel resistance-associated polymorphisms in *cqm1* gene. The $cqm1_{REC}$ was detected in the four non-treated populations surveyed at frequencies from 0.001 to 0.017 and sequence analysis from these samples revealed a novel resistant allele ($cqm1_{REC\text{-}D16}$) displaying a 16-nt deletion which is distinct from the 19-nt deletion associated with the $cqm1_{REC}$ allele. Yet a third resistant allele ($cqm1_{REC\text{-}D25}$), displaying a 25-nt deletion, was identified in unrelated samples from a *B. sphaericus* treated area. A comparison of the three deletion events revealed that all are located within the same 208-nt region amplified during the screening procedure. They also introduce equivalent frame-shifts in the sequence and generate the same premature stop codon, leading to transcripts encoding truncated proteins which are unable to locate to the midgut epithelium. The populations analyzed in this study contained a diversity of alleles with mutations disrupting the function of the corresponding Bin toxin receptor. Their locations reveal a hotspot that can be exploited to assess resistance risk through DNA-screening. (Support: CNPq Brazil- grant 403488/2008; FACEPE Brazil -grant APQ 0427-2.13/08).

Contributed Papers Bacteria 2 Tuesday, 8:30 **46**

Resistance mechanisms of *Galleria mellonella* (Lepidoptera, Pyralidae) larvae under selection by bacteria *Bacillus thuringiensis*

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Greater wax moth, *Galleria mellonella* was selected for resistance to bacteria *Bacillus thuringiensis* ssp *galleria (Bt)* in laboratory conditions. After five and ten generation the selected insects had increased resistance to Bt. The encapsulation rate has been depressed in selected larvae as compared to the control insects. However selected insects F5 and F10 had significantly increased the midgut esterase activity as compared with insects of control line. In addition, differences in redox balance of midgut (ROS generation, activity of superoxide dismutase, glutathione-S-transferase, catalase, concentration of thiols and malondialdehyde) between insects of selected line (F5) and control line were detected. These results will be discussed as some of the trade-off and resistance mechanisms of insect to *Bt* during selection under bacterial treatment.

Contributed Papers Bacteria 2 Tuesday 8:45 **47 STU**

Potential of resistance to *Bacillus thuringiensis* in a greenhouse population of *Ostrinia nubilalis* (Hübner)

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The European corn borer, *Ostrinia nubilalis* is the major pest of corn in the temperate climates, and has been effectively controlled by Bt maize since 1996. The polyphagous behavior of this pest could make it a serious threat for other crops than maize, including vegetables and ornamental plants, which cultivations could be protected by Bt-based sprays. We detected commercial greenhouses in Southeastern Spain where *O. nubilalis* became the primary pest of pepper despite repeatedly applying Bt-based spray treatments. Results of laboratory bioassays showed that the susceptibility to the Cry1 protoxins was slightly lower in this field population (5-fold) when compared with a susceptible laboratory colony. However, the differences between the two populations reduced when the activated toxins were tested, and no differences appeared when functional mortality was compared. Susceptibility to the Cry2Aa protoxin did not differ between the two populations, though the field population was more susceptible to the activated protein (3-fold). No differences were evident when the standard product HD-1-S-2005 was tested. These data pointed out that control failure in the greenhouses could be a consequence of intraspecific variability of this species instead of a resistance selection process. Laboratory selection experiments with activated Cry1Ab toxin was undertaken with a sample of the field population. The highest level of resistance occurred at generation 3 (8-fold) but the population collapsed at generation 10, after a toxin concentration increase. These results further confirm that low levels of resistance, not enough for surviving to high doses of toxin, are common in some *O. nubilalis* populations.

Contributed Papers Bacteria 2 Tuesday, 9:00 **48**

Specific binding of radiolabeled Cry1Fa toxin from *Bacillus thuringiensis* in susceptible lepidopteran species and resistant diamondback moth

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For the control of insect pests, several plant species have been transformed to express *cry* genes from *Bacillus thuringiensis*. Currently, transgenic varieties of corn and cotton, expressing Cry1Fa alone or in combination with Cry1A are being commercialized. The understanding of the mode of action of such proteins can help management of insect resistance. Indeed, the best-documented mechanism of resistance involves alterations of Cry toxins receptors. In the present work, Cry1Fa was successfully radiolabeled with [^{125}I]-Na. Quantitative binding studies were performed using brush border membrane vesicles (BBMV) from some agronomically important insect pests, including *Plutella xylostella*. The results showed the occurrence of high affinity binding sites in the insects tested. Moreover, binding analyses were carried out with BBMV from a *P. xylostella* strain (NO-QA), resistant to the *B. thuringiensis* commercial product Dipel and cross-resistant to Cry1Fa. The results showed that BBMV from resistant insects lacked their capacity to bind Cry1Fa. This result is in agreement with the binding site model previously proposed based on binding studies with susceptible *P. xylostella* insects, for which Cry1A and Cry1Fa was shown to share a common receptor. This is the first time that radiolabeled Cry1Fa has been successfully used in quantitative binding analyses and the first time that lack of binding has been shown for Cry1Fa in resistant insects. The present work confirms the value of the binding site models obtained with susceptible insects of a given species as a predictive tool of receptor-based mechanisms of resistance and cross-resistance when direct assay of resistant insects of that species is not possible.

Contributed Papers Bacteria 2 Tuesday 9:15 **49**

Mechanism of field-evolved resistance to transgenic Bt corn in *Spodoptera frugiperda*

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One of the main issues related to the increased adoption of transgenic crops producing toxins from *Bacillus thuringiensis* (Bt crops) is the potential for development of insect resistance. While a number of cases of field-evolved insect resistance to Bt crops have been reported, the specific mechanisms involved are unknown. We report on the characterization of the mechanism responsible for high levels of field-evolved resistance to transgenic maize expressing the Cry1Fa toxin. We previously reported that resistance in these insects was associated with reduced toxin binding. In this presentation we examine the potential role of this alteration in resistance considering the current Cry toxin mode of action model.

Contributed Papers Bacteria 2 Tuesday 9:30 **50 STU**

Fitness costs in *Spodoptera frugiperda* with field-evolved resistance to Bt corn

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Increasing adoption of transgenic crops expressing *cry* toxin genes from *Bacillus thuringiensis* (Bt) represents an augmented risk for development of insect resistance. Current regulatory mandates that attempt to reduce the risk of resistance evolution were developed based on models and data obtained from laboratory-selected insects. However, in order to develop a more efficient regulatory framework, we must understand how field-evolved resistance may develop and the existence of potential fitness costs in resistant insects. In this work, we determined fitness costs associated with resistance to Bt corn in a strain of the fall armyworm (*Spodoptera frugiperda*) displaying

high levels of field-evolved resistance. We performed studies to monitor fitness variables in susceptible (Ben) compared to resistant (456) strains of *S. frugiperda* when exposed to artificial diet or the corn isoline to transgenic Bt corn. In addition, we monitored performance of larvae from crosses between susceptible and resistant moths. Resistant insects exhibited lower larval and pupal mass and increased developmental time compared with susceptible and larvae from the 456 x Ben crosses. In addition, it was observed that resistant insects exhibited lower fecundity and fertility than the susceptible larvae. Our results support the existence of important fitness costs affecting fertility and development.

Contributed Papers Bacteria 2 Tuesday 9:45 **51**

Resistance of western corn rootworm to Bt maize

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The western corn rootworm, *Diabrotica virgifera virgifera,* is a major pest of maize in the United States and is currently managed by planting maize that produces insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt). During the summers of 2009 through 2011, we sampled western corn rootworm populations from fields throughout Iowa in response to complaints by growers of injury to Bt maize. Eggs collected from these populations were used to conduct laboratory bioassays. Neonate larvae from each population were assayed against two Bt hybrids, one producing Cry3Bb1 and another producing Cry34/Cry35Ab1. Larvae also were evaluated on the non-Bt near isogenic hybrid of each Bt hybrid. Larval survival was measured after 17 days. Populations from fields with a history of cultivation of Cry3Bb1 maize had significantly higher survival on Cry3Bb1 maize in laboratory bioassays than did western corn rootworm from fields not associated with severe injury to Cry3Bb1 maize. No differences were detected for survival on Cry34/35Ab1 maize. We conducted a field experiment in 2011 in two fields identified in 2009 as harboring Cry3Bb1-resistant western corn rootworm and found that injury to Cry3Bb1 maize was higher than any of the other treatments tested, except for non-Bt maize without insecticide. Survival of western corn rootworm did not differ between non-Bt maize and Cry3Bb1 maize. These data highlight the challenges surrounding management of western corn rootworm with Bt maize in continuous maize fields, and underscore the need for sound integrated pest management when applying Bt maize to manage western corn rootworm.

Contributed Papers Tuesday 8:00-10:00

Viruses 1 *Biocontrol and Biotechnology*

Contributed Papers Viruses 1 Tuesday 8:00 **52**

Effects of adjuvants on pathogenicity of *Plutella xylostella* granulovirus (*PlxyGV*) on diamondback moth (L.) (Lepidoptera: Plutellidae)

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Plutella xylostella granulovirus (*PlxyGV*) infecting the most serious pest of crucifers throughout the world, diamondback moth (DBM) *Plutella xylostella,* was susceptible to ultra violet radiation (UV). The virus lost almost all of its pathogenicity after 7 hours of exposure to UV-B radiation under laboratory conditions. The virulence of UV treated *PlxyGV* was reduced to 19.64%, 41.53%, 63.17%, 70% and 89 % after 5, 15, 30, 60 and 120 minutes of exposure to UV radiation, respectively, when compared to non-treated *PlxyGV*. Incorporation of adjuvants, Tinopal, molasses, lignin and skimmed milk, separately to *PlxyGV* suspension significantly improved residual activity of *PlxyGV* after exposure to UV radiation. These adjuvants were able to maintain the residual activity of *PlxyGV* by 31.35 to 67.78%. The molasses and Tinopal at different virus concentrations before exposure to UV light significantly increased the residual activity. Molasses showed greatest effects on the larval mortality at all virus concentrations compared to those of Tinopal and lignin before exposure to UV light. The LC_{50} calculated for virus and molasses (5.2×10^4 Granules/ml) before exposure to UV light was 9.2 and 1.75 times lower than lignin and Tinopal respectively. The overall results indicated that the natural UV protectants such as molasses and lignin can be a substitute to chemical optical brighteners in virus formulation.

Contributed Papers Viruses 1 Tuesday 8:15 **53 STU**

Lethal concentration dependent interaction of a *Agrotis segetum* Nucleopolyhedrovirus and Granulovirus in mixed infections

Jörg Thomas Wennmann, Gianpiero Gueli Alletti and Johannes Alois Jehle

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Four different baculoviruses, AgseNPV-A, AgseNPV-B, AgipNPV and AgseGV, that were isolated from larvae (cutworms) of the damaging soil pests *Agrotis segetum* and *Agrotis ipsilon* (Lepidoptera: Noctuidae) are described and characterized on the molecular level. Bioassay analyses revealed that all four *Agrotis* baculoviruses are able to infect both hosts and co-infections of a single host by more than one *Agrotis* baculovirus occur. Therefore, these viruses are considered as potential biocontrol agents for the control of *Agrotis spec*. In natural epzootics co-infections between AgseNPV and AgseGV are frequently observed. Due to co-infections of hosts mainly three different types of baculovirus interactions are described: mutualism, neutralism and antagonism. In order to investigate the potential application of *Agrotis* baculoviruses in the field their interactions have to be considered on the ecological and molecular level. Here, we focus on the interaction of AgseGV and AgseNPV-B in neonate *A. segetum* larvae in simultaneous mixed infections. The lethal concentrations of 50% and 10% mortality (LC_{50}, LC_{10}) were determined for both viruses in bioassays of 7 days of duration. Then larvae were exposed to the four different combinations of lethal concentrations: LC_{50} : LC_{50}, LC_{50} : LC_{10}, LC_{10} : LC_{50} and LC_{10} : LC_{10} and the absolute outcome of AgseNPV-B and AgseGV was determined from each treatment by quantitative PCR. It was found that the replication success of AgseNPV-B was strongly influenced by the presence of AgseGV, whereas speed of killing and thus larval mortality depended on the replication success of the NPV rather than on GV. It also shows that the terms mutualism, neutralism and antagonism need to be referred to the observed parameter, i.e. whether the replication success or mortality is considered. This finding has major implications on the evolutionary co-existence of the two viruses as well as on their practical use as biocontrol agents.

Contributed Papers Viruses 1 Tuesday 8:30 **54**

A tarantula toxin causes early cell death during in vitro insect cell infection by a recombinant baculovirus

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Baculoviruses are insect viruses used as biological control agents and expression vectors for foreign proteins in insect cells and insects. One of the major problems with the use of baculoviruses as biocontrol agents is the slow speed of kill of the target insect. Several insect-specific toxins from various organisms have been introduced into the genome of baculoviruses and shown to increase the virus speed of kill. However, most of the toxins targets in the insect host are still unknown. Baculoviruses can be used as tools to understand the mode of action of insect-specific toxic peptides in insect cells. In this work, different versions of a putative insect toxin gene (BaTx) were obtained from a cDNA of the tarantula *Brachypelma albiceps* venom gland by PCR and used to construct recombinant baculoviruses. The recombinant viruses were then used to infect two lepidopteran cell lines. The different toxin versions caused wide cell death by necrosis in different levels early on infection. Ultrastructural analyses showed cytomorphological changes with plasma membrane integrity loss. The features created a rough aspect when infected cells were observed by light microscopy. The BaTx toxin resembles channel former peptides since it shows high content of basic residues (lysine and arginine), cystein residues, and beta-sheet conformation. However, its mode of action is not known. TRP-like channels are found in insects and there is evidence of the presence of these ion-transporters in intracellular membranes as well as in plasma membrane. They are sensitive to tarantula toxin and might be the BaTx target.

Contributed Papers Viruses 1 Tuesday 8:45 **55 STU**

***In vivo* monitoring of protein expression in insect cells using recombinant AgMNPV baculoviruses**

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The *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV) is a baculovirus that infects larvae of *Anticarsia gemmatalis* (Lepidoptera), a soy bean pest. Baculoviruses have the ability to form two viral phenotypes during infection and this depends on the molecular control of the host cellular machinery. It is well known that baculovirus early transcription and protein expression is driven at first by host transcription machinery that binds to immediate early promoters in the virus genome, later on transcription is driven by virus encoded RNA polymerase which binds to late and very late promoters and is capable of hyperexpression of viral proteins, coupled with the replication of the viral genome. In this work, we generated recombinant AgMNPVs by homologous recombination, containing the *Photinus pyralis* firefly Luciferase (FLUC) gene being driven by *ie-1*, *vp39, gp64, p6.9, p10* and *polh* promoters. We also developed a method to quantify the luminescence derived from the FLUC protein produced in real time infection of insect cell lines. Here we present the profile of protein expression driven by the individual promoters during infection of UFL-Ag-286 cells, *ie1* and *gp64* promoters were first detected at 1 hour post infection (hpi), *vp39* at 4 hpi, *p6.9* at 7 hpi and *polh* at 9 hpi, with different rates of expression. This accurately display the ability of the promoter sequences to effectively control timing and amount of protein expression during infection, which gives insights into host-pathogen interactions at the molecular level and optimum timing for protein expression using baculovirus expression vectors.

Contributed Papers Viruses 1 Tuesday 9:00 **56 STU**

Host impact on *Anticarsia gemmatalis* multiple nucleo-polyhedrovirus production

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The baculovirus AgMNPV is one of the most widely used in the biological control of pests. To satisfy this application it is necessary to multiply the viruses in cell lines or larvae. The first option involves having susceptible cells that grow in laboratory conditions. For instance, AgMNPV replicates in UFL-Ag-286 (derived from *Anticarsia gemmatalis*), Hi-5 (derived from *Trichoplusia ni*) and Sf9 (derived from *Spodoptera frugiperda*) cells with different productivities. Because these cells were established from different species of larvae, there may be a host effect on the virus phenotype produced there. With the aim to study these potential host effects, AgMNPV was multiplied in UFL-Ag-286, Hi-5 and Sf9 cells. Yields were estimated by BV and OB quantification by standard methodologies. The highest BV and OB production was in UFL-Ag-286 cell, followed by Hi-5, and the lowest productivity was in Sf-9 cells. The virus progenies were analyzed by optic and electron microscopy, and yields were estimated by BV and OB quantification by standard methodologies. The quantity of nucleocapsids in the ODV was estimated by qRT-PCR. On the other hand, the structural proteomes were studied by two-dimensional gel electrophoresis, and the infectivity properties of obtained OBs were tested in UFL-Ag-286 cells and larvae in order to determine, not only the productivity, but also the viability of the viral progeny.

Contributed Papers Viruses 1 Tuesday 9:15 **57**

Growth of the UFL-AG-286 cell line and replication of the *Anticarsia gemmatalis multiple nucleopolyhedrovirus* in a new medium free of animal protein hydrolysates

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The regulations governing biotechnology processes become increasingly restrictive in relation to the use of raw materials of animal origin. These restrictions also apply to processes based on insect cell cultures. Several serum-free media are available to cultivate insect cell lines, but almost all of them still contain components of animal origin. UNL-10 is a serum-free medium specifically designed to support the growth of the UFL-AG-286 cell line, as well as the production of occlusion bodies (OBs) of the *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV). UNL-10 still contains a mixture of animal protein hydrolysates. The aims of this work were to evaluate the ability of several plant protein hydrolysates to replace animal protein hydrolysates, to design a new medium free of animal protein hydrolysates, to adapt the UFL-AG-286 cell line to this medium and to evaluate the replication of AgMNPV. A plant protein hydrolysate was selected because of its ability to promote the growth of UFL-AG-286 cell cultures in UNL-10 medium deprived of animal protein hydrolysates. UFL-AG-286 cells were adapted and maintained over 200 passages in a new medium where animal protein hydrolysates were replaced by a unique plant protein hydrolysate. Growth and metabolic parameters of static cultures adapted to the new medium were similar to those determined in UNL-10 medium. Yields of budded virus and OBs as high as $4x10^8$ $TCID_{50}$/ml and $1x10^8$ OBs/ml, respectively, could be reached in static cultures of UFL-AG-286 cells adapted to the new medium free of animal protein hydrolysates.

Contributed Papers Viruses 1 Tuesday 9:30 **58**

Towards a feasible process for the large scale production of Oryctes virus in DSIR-HA-1179 insect cell cultures

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One of the most sensitive issues in modern agriculture is the delicate balance between pest control and the danger of environmental damage by chemical pesticides. The Rhinoceros beetle (*Oryctes rhinoceros L.)* is an economically important pest of coconut and oil palms throughout Southeast Asia and the Pacific Islands. Its control using chemical pesticides has had limited success. An alternative solution is the use of environmental-friendly biological insecticides with a narrow host range. The *Oryctes virus* (OrV), a natural pathogen to the Rhinoceros beetle, has been successfully used to control this pest, but its production in infected larvae presents several disadvantages. *In vitro* production of OrV in a susceptible cell line is an attractive option for mass-producing the virus. One of the key factors in the production process is the selection of a culture medium capable of supporting cell growth to high densities and high virus yield. The DSIR-HA-1179 insect cell line was adapted to grow in three commercially available culture media, TC-100, Sf-900II and IPL-41, each supplemented with 10% serum, and cell growth and infection parameters assessed. While all culture media yielded comparable cell densities after 14 days of culture (2.0 x10^6 viable cells/ml), cells cultivated in Sf-900II produced the highest virus titer (4.3 x 10^7 TCID50/ml), followed by IPL-41 (3.2 x 10^7 TCID50/ml) and TC-100 (2.0 x 10^7 TCID50/ml). These findings, the first to be reported for DSIR-HA-1179 cell line, could be an important step towards the design of a feasible process to produce OrV at an industrial scale.

Contributed Papers Viruses 1 Tuesday 9:45 **59**

Baculovirus deleted for *chitinase*, *cathepsin* and *p10* genes improves rAAV8 vector integrity and infectiosity

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The baculovirus production system (wild type-backbone) is currently used to produce GMP grade rAAV therapeutic vectors. In this work, we have developed an AcMNPV baculovirus inactivated for the *chitinase*, *cathepsin* and *p10* genes for rAAV8 production. The rAAV8 vectors produced displayed increased integrity due to a reduced level of capsid protein degradation. The comparison with rAAV8 vectors produced with the normal, wild type bacmid, resulted in the identification of one baculovirus Cathepsin protease related cleavage site in the VP1 protein sequence along the kinetic of action of the Cathepsin during the production and purification process. Finally, *in vivo* evaluation of rAAV8 vector produced after inactivation of the Cathepsin protease has shown an increased potency (infectivity) level of the rAAV vector. The results will be discussed in view of the use of rAAV vectors for the treatment of neuromuscular diseases by *in vivo* gene therapy.

Symposium IV Tuesday 10:30-12:30
Diseases of Beneficial Invertebrates Division

Global bee health and specific issues in Latin America

Symposium IV Tuesday 10:30 **60**

Colony collapse occurrence in Africanized honey bees in Brazil

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CCD (Colony Collapse Disorder) was first reported in the United States during the winter of 2006/07; since then, annual losses are drastically affecting the U.S. pollination industry. In Brazil we observed CCD-like symptoms in Africanized honey bees in the northern region of São Paulo state on two occasions in areas where insecticides are normally applied (fipronil and other neonicotinoids). Affected colonies showed: considerable reserves of honey and pollen, no dead adult bees, and no noticeable brood diseases. In the first event honeybees disappeared from their hives in less than 15 days between two inspections; in the second event, the bees disappeared from their colonies within three days. Possibly, in this case, there was an abnormal absconding event. We observed an anomalous brood disease in this second case, with brown larvae and pupae with a small and flattened abdomen. Some adult bees were seen crawling in front of the colonies, with very low *Nosema ceranae* spore leves. Viruses including DWV, BQCV and ABPV were detected in some of this brood. We lost 70 % (14 colonies) of our experimental colonies in this second event. Between January and April 2011 we lost another 25% (5 colonies), with some forager bees showing high numbers of spores of *N.ceranae*, 40 billion/forager bee. In both these areas, various bee viruses, including IAPV, APV, DWV, and BQCV have already been detected. *Varroa destructor* is present in all colonies analyzed. Research supported by CNPq/MAPA/FAPEMIG (BRAZIL).

Symposium IV Tuesday 11:00 **61**

Status of pathogens and other potential enemies of native bumblebees in Argentina

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Honey bees and bumble bees, the two main insect pollinators, are suffering population declines in several areas of the world since *ca.* 10 years ago. Especially some species of genus *Bombus* are becoming threatened in Europe and North America, and parasites appear like one of the possible causes of these depopulations. Knowledge about parasites, pathogens and natural enemies of *Bombus* spp. in South America is scarce. Researchers from three Argentine institutions [Arthropods Laboratory of Mar del Plata National University, Entomological Division, La Plata Museum (MLP), and Center for Parasitological and Vectors Studies (CEPAVE)] are focused to detect and identify different diseases in *Bombus* species, with emphasis in the two more ubiquitous native ones: *Bombus atratus*, and *Bombus bellicosus*. At the moment, we have detected seven species of acari infesting *B. atratus* and *B. bellicosus* (*Kuzinia laevis*, *Kuzinia Americana*, *Scutacarus acarorum*, *Pneumolaelaps*

longanalis, *Pneumolaelaps longipilus*, *Tyrophagus putrescentiae*, and *Parasitellus fucorum*), the microsporidium *Nosema ceranae* in both species also in the native *Bombus morio*, the nematode *Sphaerularia bombi* in *B. atratus*, and larvae of Tachinidae flies in the same host. Other entomopathogenic protists like *Crithidia bombi* (Euglenozoa) or *Apicystis bombi* (Neogregarinorida) have been not detected in these species, but in the invasive *Bombus terrestris*. We envisage cooperative studies to further assess the diversity of pathogens and parasites, as well as their impact on native pollinators.

Symposium IV Tuesday 11:30 **62**

Epidemiology of Tetracycline resistant strains of *Paenibacillus larvae,* the cause of American Foulbrood, in the Americas

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American Foulbrood of honeybees (AFB) is the most devastating bacterial disease affecting honeybee brood worldwide and is caused by the spore-forming Gram positive bacterium *Paenibacillus larvae.* In most American honey-producing countries, the antibiotic oxytetracycline (OTC) has been used by beekeepers for decades to prevent and control AFB in honeybee colonies as an alternative to the burning of infected beehives in areas where disease incidence is high. However, tetracycline-resistant (Tc^R) and oxitetracycline-resistant (OTC^R) *Paenibacillus larvae* isolates have been detected in USA, Canada and Argentina. Resistance to tetracycline is mainly due to the acquisition of Tet determinants frequently associated with mobile elements. Horizontal gene transfer of genetic information between bacterial cells is an integral factor in the generation of genetic variability and evolution in bacteria. *Paenibacillus* larvae highly-resistant phenotypes have been correlated with the presence of natural plasmids carrying different tetracycline resistance determinants in North America and intermediate and induced resistant strains in South America. A summary of recent knowledge about tetracycline resistance in *P. larvae* populations and the potential for transferring tetracycline and/or oxytetracycline resistance determinants between *P. larvae* strains and other *Paenibacillus* and *Bacillus* species will be presented and discussed.

Symposium IV Tuesday 12:00 **63**

Molecular pathogenesis of American Foulbrood, a globally occurring epizootic of honey bees

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The etiological agent of the globally occurring epizootic American Foulbrood (AFB) of honey bees is the gram-positive bacterium *Paenibacillus larvae* (*P. larvae*). Despite being one of the most important honey bee pathogens, the pathogenesis of *P. larvae* infections is still poorly understood hampering the development of sustainable control or curative measures. The existence of different genotypes of *P. larvae* which differ in virulence opened the possibility to explore the virulence mechanisms by simply comparing these genotypes using different –omics approaches. Comparative genomics using suppression subtractive hybridization (SSH) and comparative proteomics via 2D-SDS-PAGE analysis led to the identification of several putative virulence factors including various toxins, secondary metabolites, proteases, and an S-layer protein. The functional analysis of some of these putative virulence factors will be presented and their role in pathogenesis and their impact on virulence will be discussed.

Symposium V Tuesday, 8:00-10:00

Nematodes Division

EPN Discovery and Implementation in Latin America: Current Research and Future Directions

Symposium V Tuesday 10:30 **64**

Current status on the discovery and implementation of EPN in Brazil and Argentina

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Entomopathogenic nematodes (EPN) have great potential for biological control of insect pests of agricultural importance. They have a broad host range and are safe to the environment. In this respect, South American countries have a great opportunity to develop and implement the use of EPN. About eight countries in this continent are currently conducting research on this group of nematodes. Most research has focused on biotic surveys, taxonomic descriptions of species and isolates as well as studies on their biological and ecological traits including laboratory testing of endemic and exotic species/isolates against target insects. Moreover a few countries have initiated studies on EPN mass production mostly for laboratory and small scale application. In this presentation, I will review research conducted in Brazil and Argentina, two countries with a considerable diversity of EPN species, but quite contrasting trajectories in the implementation and development of this group of entomopathogens in pest management.

Symposium V Tuesday 11:00 **65**

Entomopathogenic nematodes in Venezuela: A short history with a promising future

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The study and utilization of entomopathogenic nematodes (EPN) in Venezuela have been neglected for many years. Main causes are constituted by the lack of specialists, financial resources and governmental policies directed to include these organisms in integrated pest management programs. However in the last 5 years, some advances have been achieved and nowadays there are at least 2 laboratories with specialized personnel which have made significant advances implementing nematodes as biological control agents. Venezuela is considered as “mega-diverse” country in terms of biodiversity, but sadly there is no a national plan for sampling, collection and evaluation of EPN with pesticide potential; although some areas have been sampled intensively and more than 30 different strains are kept in our laboratories, some of them considered new species. The utilization of EPN have been restricted to laboratory and field experimental levels with an emphasis in controlling fruit flies (*Anastrepha*) in guava, sapodilla and mango orchards, with very promising results; and it is possible to start delivering EPN in commercial fields in the next 2 years. The most important limitation for mass production is the cost of the raw material for rearing the symbiotic bacteria of the nematodes, which is too expensive for Venezuelan standards, however, this year tests in alternative materials have been started to tackle down the production costs in order to offer in the next 5 years a powerful and economic option to the Venezuelan farmers.

Symposium V Tuesday 11:30 **66**

Development and use of entomopathogenic nematodes in Cuba

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In Cuba, the entomopathogenic nematodes (EPN) are use since the 80s in last century and represent one of the most commonly biological control agent used for pest management. Species belong to *Steinernema* and *Heterorhabbditis* genera are present, but *Heterorhabditis bacteriophora* Poinar is the only one specie used in field. This nematode is producing by *in vivo* method (with *Galleria mellonella*) in more than 50 Mass Rearing Laboratories for Biological Control Agents, producing more than 672 442,2 millions of juveniles in 2010. The EPN are used in rice, citrus, sweep potato, coffee, pineapple, cabbage, vegetables, ornamental plants, corn and banana for management pest like *Lissorhoptrus oryzophilus*; *Pachanaeus litus*, *Cylas formicaruis*, *Hypothenemus hampei; Phyllophaga* spp., *Plutella xylostella*; *Agrotis* spp.; *Atta insularis*; *Spodoptera frugiperda and Cosmopolites sordidus* respectively. During several years, the EPN has been used as substitute for chemical pesticides, but Cuba is now involved in transformations their agricultural system (land use, technologies and pest management) for increasing food supply. Improving agro-ecological management of crops and pest, including research for enhanced the use of biological control agents; represent challenges for researches and farmers. We present the development route of EPN in our country and some examples of results in cabbage, sweep potato and coffee.

Symposium V Tuesday 12:00 **67**

Perspective and research of Entomopathogenic Nematodes in Chile

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The first reference of entomopathogenic nematodes (EPN) was in 1957, when the strain DD-136 (*Steinernema carpocapsae*) was field released by Dr. S. R. Dutky in the south of Chile to control white grubs. Thus, Chile was the first non-U.S. country to work with EPN. Unfortunately, this pioneer job was abandoned by the sudden appearance of the DDT. The works on EPN were reinitiated in 1997 by a USDA-INIA (Chile) cooperation to look for these organisms in the Eastern Island, rendering only Diplogasterid nematodes. Later, in 2006, scientist from CABI International and INIA (Chile) began a 3 year survey under the Darwin Initiative program. 1,440 soil samples were collected along the country, yielding a 7% of positive samples for EPN. Three species of *Steinernema* and two *Heterorhabditis* were identified, including two new species of *Steinernema* (*S. australe* and *S. unicornum*) and one of *Heterorhabditis* (*H. atacamensis*). The later named after a sample collected in the Atacama Desert. This nematode collection has been screened against several agricultural pests, such as Apple moth (*Cydia pomonella*), Grape weevil (*Naupactus xanthographus*), Raspberry weevil (*Aegorhinus superciliosus*), Fuller's rose weevil (*Asynonychus cervinus*), Black vine weevil (*Otiorhynchus sulcatus*), Tebo worm (*Chilecomadia valdiviana*), and Ghost moth (*Dalaca pallens*). Experimental data showed an average of 70% control in field applications, against the larval stage of the target pest. However, the commercial use of EPN in Chile is limited, mainly due to the lack of massive production, and remains as the principal limitation for the utilization of this biological control alternative.

Contributed Papers Tuesday, 10:30-12:00

Fungi 2

Contributed Papers Fungi 2 Tuesday 10:30 **68**

Proteomic analysis of native strains of *Beauveria bassiana* and *Metarhizium anisopliae* and their toxicity against soybean weevil

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In Mexico soybean weevil (SW) *Acanthoscelides obtectus* is an important pest, so we are looking for native isolates fungi and their potential as biocontrol agents. Ten isolates of *Beauveria bassiana* and *Metarhizium anisopliae* were selected to use the extracellular secretion obtained in a culture medium containing 1 % of SW powdered (induced medium), and medium non induced. Isolates *Bb* AGG24 and *Ma* AGG28 presented the highest pathogenicity, causing 84.9 and 64% of insects mortality, with CL_{50} of $9.2x10^4$ conidia/mL and TL_{50} of 2 days, while Ma AGG28 had an CL_{50} of $1.6x10^6$ conidia/mL and 2.7 days. To characterization of the extracellular proteins secreted four protein extraction methods were evaluated, determining that the precipitation with ammonium acetate 0.1 M in absolute methanol was the most efficient in terms of resolution and quality (2D-SDS-PAGE).Protein characterization results using the GelScape revealed 40 clear spots in secretions of *B. bassiana*, pH 5.6 to 7.5 and a molecular mass from 19 to 102 kDa, while to *M. anisopliae* spots were observed to pH of 4.4 to 9.3, with a weigh of 15 to 120 kDa According with the isoelectric points and molecular weights, ten proteins were identified (Swiss-EBI) as implicated with the mechanisms of toxicity against this pest .

Contributed Papers Fungi 2 Tuesday 10:45 **69**

A 1,4-benzoquinone reductase of the entomopathogenic fungus *Beauveria bassiana* is involved in the degradation of *Tribolium castaneum* defensive secretions

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Tribolium castaneum, a major pest of stored and processed grains, is a poor host for the entomopathogenic fungus *Beauveria bassiana,* rendering biological control efforts at using the fungus against the beetle problematic. Glandular alkyl-1,4-benzoquinones (BQs) are the major components of the defensive secretions produced by *T. castaneum*. In this work, a 1,4-benzoquinone reductase (*bqr*) gene from *B. bassiana* was characterized, and its function in relation to BQ degradation probed. A cDNA clone corresponding to *bqr* was isolated and characterized. The ORF consisted of 947 nucleotides and encoded a deduced protein of 201 amino acids. Reduced germination, and a significant inhibition on *B. bassiana* growth were observed when the fungus was incubated in culture media containing *T. castaneum* gland extracts or synthetic BQs. Below the minimal inhibitory concentration, *bqr* expression was significantly induced in BQ-exposed fungi, suggesting a role for the protein in detoxifying BQs. The largest gene induction and enzyme activity were observed using 2 and 1 □g/□l of BQ, respectively. A *bqr* targeted gene disruption mutant of *B. bassiana* was constructed and a *bqr* overexpressing strain was obtained. The targeted gene knockout strain lacking *bqr* displayed a slightly decreased virulent phenotype against *T. castaneum,* whereas *B. bassiana* overexpressing the *bqr* gene resulted in significantly higher mortality rates (> 2-fold) as

compared to the wild-type parent strain. These results shed light on the interaction between entomopathogenic fungi and tenebrionid defensive secretions, suggesting a novel function for a fungal quinone reductase as a specific virulence factor against quinone-secreting tenebrionids.

Contributed Papers Fungi 2 Tuesday 11:00 **70**

Characterization of a hydrophobin gene promoter for efficient gene expression in *Beauveria bassiana*

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To search a desired promoter for genetic improvement of fungal biocontrol agents, a 1798-bp promoter (Phyd1) upstream of the *hyd1* gene coding *Beauveria bassiana* Class I hydrophobin was characterized for the first time by upstream truncation and site-directed mutation. Truncating Phyd1 to -1290 bp caused 1.6-fold increase of transcriptional expression of *eGFP* gene (coding enhanced green fluorescence protein) in transgenic *B. bassiana*. This truncated promoter (Phyd1-1290) with three transcription factors (Mat-Mc at -1066 bp, NIT_2 at -626 bp and StuA at -201 bp) drove *eGFP* expression 15.6-fold more efficiently than PgpdA, a promoter widely applied for gene expression in fungi. Under its control, *eGFP* was expressed in condiogenic cells and conidia much better than in younger mycelia during 7-day growth of transgenic colonies at 25°C. Further truncating Phyd1-1290 to -1179, -991 and -791 bp reduced *eGFP* expression by 16.7, 71.3 and 98%, respectively. The *eGFP* expression was significantly reduced by the site-direction mutation of Mat-Mc (17%), NIT_2 (52%) or StuA (81%) replaced with a *Hin*dIII restriction site. Conclusively, Phyd1-1290 is an excellent promoter to drive gene expression in *B. bassiana* conidia often formulated as active ingredients of mycoinsecticides.

Contributed Papers Fungi 2 Tuesday 11:15 **71**

A Class III histidine kinase gene (*BbHK1*) regulates conidiation in entomopathogenic fungi *Beauveria bassiana*

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Two-component signaling pathway is a very conservative signal transduction route in fungi and includes histidine kinases, response regulators, and sometimes phosphotransfer proteins. Genomic sequence analyses have revealed the presence of multiple histidine kinases in fungi and all histidine kinase genes could be categorized into 11 classes. The fungal members of Class III histidine kinases have were previously shown to mediate osmoregulation and resistance to dicarboximide, phenylpyrrole and aromatic hydrocarbon fungicides. In this study, the gene encoding histidine kinase gene in *B. bassiana* (*BbHK1*) was identified and catalogued into Class III. Use the homologous recombination method, the gene knockout mutant was constructed. Phenotype analysis revealed that the knockout mutant had very poor ability to conidiation and conidia yield decreased about 90%. The lost conidiation ability of mutant could be restored by introduction of a whole *BbHK1* gene. These results confirmed the crucial role of BbHK1 in conidial development and suggested that this pathway be a potential target for improving conidia production of biocontrol agents.

Contributed Papers Fungi 2 Tuesday 11:30 **72**

The *Beauveria bassiana* gene *Bbpmr1* is important for cation homeostasis, conidiation, multi-stress tolerance and virulence

Jie Wang and Ming-Guang Feng*

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The *pmr1* gene of *Beauveria bassiana*, encoding the P-type Ca^{2+}/Mn^{2+} ATPase, is a homologous to that of *Saccharomyces cerevisiae*. The *pmr1* knockout strains (*Δpmr1*) exhibited hypersensitivity to EDTA and grew very slowly when Mn^{2+} or Ca^{2+} was removed from the medium. Some other tested phenotype of *Δpmr1* differed significantly from those of wild type and *Δpmr1*/pmr1, which were similar to each other. Significant phenotypic changes in knockout mutant included decreased conidial yields, lower tolerance to environmental stresses (including thermal, oxidative and osmotic stress), and reduced virulence to first-instar larvae of *S. litura*. Moreover, the *Δpmr1* mutant became hypersensitive to cell wall synthesis inhibitor (Congo red) and the microtubule depolymerizing drug (Carbendazim). These results confirmed the crucial role of *pmr1* in cation homeostasis, cell wall integrity and associated phenotypes important for the fungal biocontrol potential.

Contributed Papers Fungi 2 Tuesday 11:45 **73**

The cell wall integrity in entomopathogen *Beauveria bassiana* depends on mitogen-activated protein kinase kinase signaling pathway

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Gene *Mkk1*, a member of mitogen-activated protein kinase kinase (MAPKK), was reported to play an important role in cell wall integrity pathway in fungi. The mkk1 gene in *Beauveria bassiana* has been successfully cloned and its function was analyzed by generating knockout mutant via target replacement method. The results showed that the gene disruption caused significant physiological changes in mutant, including decreased tolerance to Congo red (CR), Sodium dodecyl sulfate (SDS) and carbendazim, mild decreased tolerance to NaCl, ethirimol and tricyclazole, and decreased tolerance to thermal stress and UV-B resistance in conidia, as well as decreased conidial virulence to the larvae of the oriental leafworm moth *Spodoptera litura*. Compared to the wild type strain, the *Δmkk1* mutant had decreased ~90% in expression level of the two plasma membrane sensors of the Wsc-family (*wsc1* and *wsc2*), and its transcription of two chitin synthase genes (*chs2* and *chs8*) reduced to ~50% under the stress caused by CR. These results confirmed the crucial roles of *mkk1* in regulating the cell wall integrity pathway and associated functions important for the fungal biocontrol potential.

Contributed Papers Tuesday, 10:30-12:15

Microbial Control 1

Contributed Papers Microbial Control 1 Tuesday 10:30 **74 STU**

Toxicity of Cry1 and Vip3A proteins to *Diatraea saccharalis* (F, 1794) (Lepidoptera: Pyralidae) and binding to brush border membrane vesicles

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Some varieties of maize are being engineered with *cry1* and *vip3A* genes from *Bacillus thuringiensis* in order to protect them from several lepidopteran species. Therefore, the study of the insecticidal activity of Cry and Vip proteins on the most important pests of this crop, as well as their compatibility regarding lack of cross-resistance, is of great interest. In the present study, a Brazilian population of *Diatraea saccharalis* has

been tested for susceptibility to Cry1Aa, Cry1Ac, Cry1Ca and Vip3Aa by artificial diet surface contamination. The Vip3Aa protein showed a toxicity (LC_{50} value around 125 $ng.cm^{-2}$) that was higher than the ones obtained with Cry toxins (LC_{50} ranged from 193 to 785 $ng.cm^{-2}$). Biotinylated Cry1Aa, Cry1Ab, and Cry1Fa proteins showed specific binding to the midgut brush border membrane vesicles of the larvae. Heterologous competitive binding assays suggested a model of 2 receptors; a common receptor for Cry1Aa and Cry1Ab another one for Cry1Fa and Cry1Ab. Vip3Aa did not compete for binding with any of the Cry proteins tested. The high levels of toxicity of the Vip3Aa protein to *D. saccharalis* and the lack of shared receptors with Cry proteins make it an ideal tool for controlling this pest.

Contributed Papers Microbial Control 1 Tuesday 10:45 **75 STU**

Cool Caterpillars: Low temperature biological control of a climbing cutworm.

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The growing season for many pests is during the summer where temperatures are often high; however, not all pests conform to this pattern. *Abagrotis orbis* is a climbing cutworm pest of vineyards in the Okanagan Valley. Eggs are laid and hatch in the fall and larvae overwinter as early instars. In the spring, the larvae begin to climb the grape vine and cause considerable damage by consuming the nascent buds. Thus the only windows for controlling this pest are in the fall and spring when temperatures are relatively cool. The optimal development temperature for *A. orbis* is 15°C, and therefore it is important to find microbial control agents that operate effectively at this temperature. Here we screen a range of commercially available microbial control agents. We tested *Metarhizium brunneum* (F52), *Beauvaria bassiana* (GHA), *Bacillus thuringiensis* kurstaki (Dipel 2X), *Heterohabditis megedis*, *Steinernema feltiae,* and *Steinernema kraussei* in separate laboratory bioassays against *A. orbis* 1st and 3rd larvae at three temperatures: 10°C, 15°C, and 20°C. We found temperature and concentration effects, but no interactions. Both fungi achieved over 60% mortality at 10°C. Though not recommended for this organism, the nematodes were able to complete their lifecycle in *A. orbis*. *H. megedis* caused 50% mortality at 20°C, but at 10°C there was less than 15% mortality. *S. kraussei* achieved over 60% mortality at 20°C and over 30% mortality at 10°C. The results indicate that either of the fungi will be suitable for field-testing, in addition to *S. kraussei* and *S. feltiae* either singly or in combination.

Contributed Papers Microbial Control 1 Tuesday 11:00 **76 STU**

Insect-specific sodium ion pump targeting μ-Agatoxin IV peptide inhibits *Trichoderma asperellum* conidiation

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There are many disease-pest complexes in plants that can not be controlled using chemical approaches. Biological control of such complexes seems to be a rational method. *Trichoderma* species are well-known plant disease biological control agents throughout the world however there is only little information on their entomopathogenicity in litreature. In this research, two *T. asperellum* isolates were selected out from more than 100 *Trichoderma* isolates after several *in vitro* tests, and their fatal entomopathogenicity was shown on *Tribolium confusum* adults. Both isolates were genetically cotransformed with pAN7-1, and pAN52-1N expression vector harboring a specific genetic construct to coexpress both μ-Agatoxin IV peptide and green fluorescent protein encoding open reading frames. The first ORF included a signal peptide sequence instantly followed by the sequence of insecticidal peptide. The expression of green fluorescent protein was observed with transformants however conidiation process was suppressed by the toxic peptide.

Contributed Papers Microbial Control 1 Tuesday 11:15 **77 STU**

Hybrid approach to the control of greenhouse whitefly in Australia

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Tritrophic interactions between pest insects and their natural enemies are common in nature but can be counter-productive in plant protection. When developing integrated pest management protocols, it is therefore important to know of interactions between the different management options available. The parasitic wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) has been shown to be compatible with some entomopathogenic fungi also used for the control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). However, the optimal timing and order of application of these two biological control agents has not been investigated. This study examined the hypothesis that *E. formosa* and entomopathogenic fungi are compatible in the integrated pest management of greenhouse whitefly, regardless of the order in which they were applied. Two species of fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Isaria fumosorosea* Wize (Deuteromycota: Hypocreales) were selected from the collection of the Queensland Department of Primary Industries. Two experiments were conducted in growth rooms at 25°C to determine the optimal application time and order of application of the two biological control agents. In the first experiment, the parasitic wasp was released prior to application of the fungus; the second experiment was conducted in the reverse order. The results of this study will enable better control of greenhouse whitefly without compromising accepted biological control agents. Future work will examine the compatibility of the entomopathogenic fungi with the Australian wasp *Eretmocerus warrae* Naumann and Schmidt and the mechanisms behind the interactions.

Contributed Papers Microbial Control 1 Tuesday 11:30 **78**

Biopesticide potential of organisms from ecological extremes

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Fungal and nematode isolates were collected from a range of habitats in southern Chile and maritime Antarctica and investigated for their insecticidal activity as well behavioural

characteristics and environmental adaptations. In southern Chile (Patagonia and Magallanes Regions) two new species of entomopathogenic nematode were discovered (*Steinernema australe* and *S. unicornum*), together with two new species of symbiotic bacteria. The insect-killing capability of these two nematodes is presently being carried out at INIA, against a range of insect pests from Chile. The surveys from maritime Antarctica revealed a number of fungal genera that had no insecticidal history but could be baited out with insects. Two of these genera are presently being investigated for growth and production characteristics at 0 to 35°C, and their insecticidal activity against a range of insects, namely *Galleria mellonella* L., *Musca domestica* L. *Tenebrio molitor* L. and *Otiorhynchus sulcatus* Fabricius. Most of these isolates grew at 0 to 25°C. Sporulation occurred at 10 and 20°C, but subsequent germination at cooler temperatures was significantly better for spores produced at 10°C, compared to 20°C. Insecticidal activity was examined via dipping, injection and by soil inoculation. Significant mortality of *G. mellonella* and *M. domestica* was observed via injection, and of *G. mellonella* via soil inoculation, dipping had no effect, however there was considerable isolate variation.

Contributed Papers Microbial Control 1 Tuesday 11:45 **79**

Paecilomyces lilacinus:* possible candidate to control the leaf-cutter ant *Acromyrmex lundii?

Daniela Goffré and Patricia J. Folgarait

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Many leaf-cutter ant species are well known pests in Latin America. To cultivate a symbiotic fungus (Basidiomycota: Agaricales) from which they feed, these social insects cut and transport a great variety of leaves, causing economically important losses in crops and plantations. Traditionally these ants have been chemically controlled, but pesticides are known for being harmful to ecosystems. An environmentally friendly strategy to reduce the number of leaf-cutter ants is biocontrol. In this work we evaluated the effectiveness of a strain of the entomopathogen *Paecilomyces lilacinus* (Ascomycota: Eurotiales), obtained from the leaf-cutter ant *Acromyrmex lundii,* to control the same species of leaf-cutter ant. Ants from six *A. lundii* colonies were individually inoculated with conidia of *P. lilacinus* at a concentration of 1 x 10^6 conidia/ml. Ants treated with water were set up as controls. Each ant was individually tracked to record the date of death. All ants treated with *P. lilacinus* exhibited a significantly lower survivorship compared to the control. We also classified all fungi that appeared on their dead bodies as internal or external in order to assign the cause of death. *P. lilacinus* was responsible for 61,8 % (49,6 – 83,0) of the mortality in inoculated ants. We found a significant negative correlation between the percentage of ants infected with *P. lilacinus* and the percentage of ants infected naturally with other entomopathogens. Although *P. lilacinus* proved to be pathogenic to *A. lundii*, the effectiveness of this strain will depend on other characteristics that are not possible to control under field conditions.

Contributed Papers Microbial Control 1 Tuesday 12:00 **80**

Can a leaf-cutter *Paecilomyces lilacinus* strain be used to control red fire ants?

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P. lilacinus (Ascomycota: Eurotiales) is a generalist entomopathogenic fungus, mostly known as a nematode pathogen. However, several strains were proven to be virulent on *Solenopsis invicta* ants in China. In this work we evaluated the virulence of a strain isolated from the leaf-cutter ant *A. lundii*, which was demonstrated to be effective against *A. lundii*. Ants from five previously disinfected *S. invicta* colonies were inoculated in batches with a conidia suspension of *P. lilacinus* at a concentration of 10^7 conidia/ml. As controls, we submerged ants in sterile water. We recorded the death of each ant daily. Afterwards, dead ants were individually disinfected and placed in humid chambers. We also identified all fungi that appeared on dead workers to assign the cause of death. Survivorship of ants innoculated with *P. lilacinus* were statistically equal to controls in four of five colonies. *P. lilacinus* was responsible for a median mortality of 19,0 % (1,5 – 34,8) in innoculated ants. The great variation observed could be explained by either the health status of the ants and/or the competitive capacity that allowed co-ocurrence with other entomopathogens previously present in the ants. From these results, we concluded that this strain of *P. lilacinus* had a minor effect on the survivorship of *S. invicta;* therefore it is not a good candidate for the control of fire ants. However, further studiesn should be encouraged for its use as a candidate for the biological control of *A. lundii* because it doesn´t affect non target ants.

Contributed Papers Tuesday, 10:30-12:30

Viruses 2 *Genomes and Transcriptomes*

Contributed Papers Viruses 2 Tuesday 10:30 **81**

Genome sequence and organization of a baculovirus isolated from *Perigonia lusca* (Lepidoptera: Sphingidae)

Fernando L. Melo[1], Daniel M. P.Ardisson-Araújo[1], Fabricio S. Morgado[1], Daniele V. Freitas[1], Miguel Andrade[1], Daniel R.Sosa-Gomez[2] and Bergmann M. Ribeiro[1]

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The complete genome sequence of a single nucleopolyhedrovirus isolated from *Perigonia lusca* (Lepidoptera: Sphingidae), an important pest of the Paraguay tea (*Ilex paraguariensis*), was determined using the pyrosequencing technique (454 Life Sciences Technology). The PeilSNPV genome is approximately 132.000 bp, with a low GC content (39.6%). Overall, it contains 141 putative open reading frames (encoding proteins with at least 50 amino acids), and unique genes were not found. Several single nucleotide polymorphisms (SNPs) were found along the genome. Interestingly, a high frequency of non-synonymous SNPs was found in the conserved structural gene *vp91*, which may affect the biological functioning of the encoded protein. Phylogenetic analysis using *polyhedrin* and *lef-8* sequences reveals that PeilSNPV belongs to the group II *Alphabaculovirus* and is related to EupsNPV, ApciNPV, ClbiNPV and OrleNPV. This was further confirmed by the presence of a typical F protein, common to all group II *Alphabaculovirus*. The elucidation of the complete genome of PeilSNPV will help to better understand the biology of this virus and may assist the establishment of an effective biological control program for *Perigonia lusca*.

Contributed Papers Viruses 2 Tuesday 10:45 **82 STU**

Ultra-deep sequencing of AcMNPV and comparison to original genome sequencing

Aurélien Chateigner, Davy Jiolle, Carole Labrousse, Annie Bézier and Elisabeth Herniou

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Natural selection relies on genetic variation to lead to ecological adaptation. Baculovirus occlusion bodies containing numerous genomes foster the maintenance of genetic diversity. This diversity, characterised by restriction endonuclease profile or gene sequencing, has long been known in AcMNPV. Here, we present a novel approach to assess the diversity harboured in the original P.Vail isolate using 150bp Illumina© paired-end ultra-deep sequencing techniques. We obtained a theoretical genome coverage superior to 190000, allowing for solid statistical tests on the genetic variation. The genome of AcMNPV clone C6 shows a 0.2% pairwise difference with our isolate consensus sequences, from which it derived. However, in our isolate we found 40 SNPs and 12 INDELs over the 133,926 bp long genome, including the variation of the C6 clone. SNPs where found in essential genes like IE, PIF and lef families, but also in auxiliary genes like pk-1and pcna, and in other non-essential genes. INDELs were found in auxiliary genes like egt, but also in essential genes like bro. This approach provides precise information on the diversity present in this isolate, with clear modulation of genes involved in essential pathways, but also in host adaptation pathways.

Contributed Papers Viruses 2 Tuesday 11:00 **83 STU**

Transcriptome analysis of the *Cydia pomonella* granulovirus

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The *Cydia pomonella* granulovirus (CpGV) is widely used for the biological control of codling moth (CM, *C. pomonella*) in apple and pear production in Europe and many other apple growing regions worldwide. In recent years observations of resistance to CpGV was made in several European countries. On the other hand, new CpGV isolates overcoming this resistance were identified and applied in orchards with resistance. To understand better the interaction between CpGV and its host and to determine the genetic factors involved in the virulence of the virus the transcriptome of the virus and the expression profile of its genome is analysed. By quantitative reverse transcription PCR (qRT-PCR) analyses and microarray studies, the specific time frame of the infection process in midgut and fatbody tissue was discovered. *In vitro* transcritpion analysis of NPVs in different cells lines had showed that the gene expressions in baculovirurses are expressed in a temporarily regulated gene cascade. *In vivo* transcription analysis of CpGV by PCR and quantitative PCR revealed a contemporary expression of early and immediate early and late and immediate late genes as well, which might be caused by a non-synchronic infection under *in vivo* conditions.

For CpGV resistance an early block in the virus replication had been proposed because replication of viral DNA could not be detected in resistant CM larvae. Strikingly, viral transcripts of all transcriptional classes, including late and very late transcripts were detected in both in susceptible CM and delayed in resistant CM individuals, indicating that the resistance in CM is not due to a complete shut down of CpGV infection. Microarray analyses are used to investigate the whole transcriptome to understand the differences in the infection process of susceptible and resistant CM larvae.

Contributed Papers Viruses 2 Tuesday 11:15 **84 STU**

Ac53, ac78, ac101 and ac103 are newly discovered core genes in the family *Baculoviridae*

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The family *Baculoviridae* is a large group of insect viruses containing circular double-stranded DNA genomes of 80 to 180 kbp, which have a broad biotechnological application such as bioinsecticide uses, systems of protein expression and as tools for gene delivery or platforms to expose antigens in vaccine formulations. A key feature to understand and manipulate them is the recognition of orthology, a biological property that reveals putative genetic composition of ancestors. However, the differences in gene content and evolutionary distances among the known members of this family make it difficult to assign sequence orthology, a knowledge necessary to better understand the characteristics of each virus species, and to address better genetic manipulation processes to ensure new biotech products. In this study, the genome sequences of 58 baculoviruses were analyzed with the aim to detect previously non-described core genes because of their remote homology. A routine based on Multi PSI-Blast/tBlastN and Multi HaMStR allowed us to detect 30 accepted and 4 non-previously described orthologous sequences in *Baculoviridae*. Our results show that the *ac53*, *ac78*, *ac101* and *ac103* genes have orthologs in all genomes and should be considered as core genes.

Contributed Papers Viruses 2 Tuesday 11:30 **85**

Nucleopolyhedrosis causing virus from the crane fly *Tipula oleracea*

Annie Bézier[1], Darren Obbard[2], Julien Thézé[1] and Elisabeth A. Herniou[1]

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In the 1950s, K.M. Smith described nucleopolyhedrosis disease, typical of baculoviruses, from cranefly (Diptera, Tipulidae) larvae. Based on the current taxonomy of the family *Baculoviridae,* the *Gammabaculovirus* genus is Diptera specific. We therefore postulated that our cranefly virus could be related to the mosquito gammabaculovirus CuniNPV. We sequenced the genome of a historical sample of dsDNA virus isolated from the cranefly *Tipula oleracea*. After whole genome amplification and Roche 454 sequencing, the assembly produced a 146 kb contig, with a GC content of 26%. Genome annotation predicted 131 protein-coding open reading frames (ORFs). Blast searches revealed only 20 ORFs are similar to the 30 baculovirus core genes. These corresponded to the set of core genes shared by baculoviruses and nudiviruses. Moreover 23 genes have homologues in other nudiviruses, indicating that our virus was not a gammabaculovirus. Phylogenomic analyses clearly confirmed that this crane fly virus belongs to the nudivirus clade. Recently, another Diptera-infecting nudivirus has been discovered in *Drosophila*. We therefore conducted comparative genomics and phylogenetic analyses to assess how these 2 viruses are related. It appears that unlike in the family *Baculoviridae*, nudiviruses infecting the same host order can be fairly distantly related.

Contributed Papers Viruses 2 Tuesday 11:45 **86 STU**

Gene acquisition convergence drives adaptation in distant insect viruses

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Many virus species from distantly related families can thrive within the same host species. Indeed, both entomopoxviruses and baculoviruses have been isolated from diseased *Adoxophyes honmai* (Tortricidae) and *Pseudaletia separata* (Noctuidae) caterpillars and mixed infections could occur in these insect populations. In theory, viruses infecting the same host species are subjected to similar immune responses from their hosts. Their genomes should bare the traces of similar adaptation. We sequenced and characterized the genomes of two entomopoxviruses (AdhoEPV and PsseEPV) with overlapping host spectrum to known baculoviruses. These genomes are phylogenetically close and with high synteny conservation to the previously sequenced AmEPV. To assess if entomopoxviruses share adaptive genes with other insect viruses, we performed an ortholog clustering, including all completely sequenced poxviruses, baculoviruses and other insect large DNA viruses as well as cellular organisms, followed by robust phylogenetic analyses to infer the evolutionary history of genes. We found adaptive convergence in distantly related viruses, with ancestral gene acquisition either from hosts, other cellular organisms or even virus. Furthermore, we found a gene in PsseEPV and in the baculovirus XecnGV, which is a clear recent adaptive convergence toward their common specific host species. Gene acquisition convergence drives adaptation in distant insect viruses and can be a potential asset for the development of biocontrol applications.

Contributed Papers Viruses 2 Tuesday 12:00 **87 STU**

Construction of an *Adoxophyes honmai* nucleopolyhedrovirus bacmid system to elucidate genes related to viral killing speed.

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The smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae), is one of the most important pests of tea plants in Japan. *A. honmai* is susceptible to two nucleopolyhedroviruses (NPVs), *A. honmai* NPV (AdhoNPV) and *A. orana* NPV (AdorNPV). These two viruses are genetically closely related, but differ in killing speed: AdhoNPV is a slow-killing virus, whereas AdorNPV kills the host quickly. To identify which gene(s) determine the killing speed of the *Adoxophyes* NPVs, we constructed an AdhoNPV bacmid system. For the bacmid system, we inserted a bacmid cassette, comprising a bacterial replication origin (mini F-replicon), an antibiotic resistance marker gene (Km^R), and a transposon element (lacZ:attTn7:lacZ), into the unique *Fse*I restriction site of the AdhoNPV genome. However, since the *Fse*I site is located within the coding region of AdhoNPV ORF31 (*Adho31*), we connected one of two *Fse*I-digested *Adho31* fragments with the bacmid cassette (Adho31-bacmid cassette), and then ligated the resultant DNA into the unique *Fse*I site of the AdhoNPV genome, to repair *Adho31*. The AdhoNPV bacmid DNA was transformed into *Escherichia coli* (HST08), after which the cells were transformed helper plasmid pMON7124. This transformed *E. coli* HST08 strain was designated as the AdhoNPV bacmid system. This system is being used to elucidate gene(s) related to killing speed in *Adoxophyes* NPVs.

WEDNESDAY 8th

Symposium VI Wednesday 8:00-10:00
Viruses and Fungi Divisions

Pathogen induced host behaviour - clues for mechanisms

Symposium VI Wednesday, 8:00 **88**

A behaviour-manipulating virus in a parasitoid wasp: genomics and transcriptomics insights

Julien Varaldi*, David Lepetit and Marie-Christine Carpentier

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The transmission of parasites is often dependent on the behaviour of their hosts. Thus, natural selection has selected genes in the parasites genomes that manipulate the behaviour of the host to their own benefit. However these genes and their functional impact on the hosts have rarely been identified. Here I will present the first step towards their identification in a system involving an inherited virus and a parasitoid wasp. The parasitoid *Leptopilina boulardi* specifically parasitizes *Drosophila* larvae and one *Drosophila* allows the development of a single parasitoid. As expected, parasitoid females usually refuse to lay supernumerary eggs in already parasitized larvae, especially when unparasitized hosts are available. However, some parasitoid lines often accept to do so (superparasitism) and this behaviour is stably inherited through generations. This behaviour is in fact caused by an hereditary DNA virus (called LbFV). The virus directly benefits from this behavioural alteration, since it allows its horizontal transmission between parasitoids sharing the same larva. To address the question of the mechanisms underlying this behavioural manipulation, we have first sequenced the genome of this virus. In addition, we have compared the transcriptome of infected and uninfected females using a combination of RNAseq and 454 sequencing on this non-model species. In this talk, I will present and discuss the very first results obtained from this work. In addition to the fundamental interest of this work, the perspective of identifying superarasitism genes may be of interest from a biocontrol perspective since parasitoids are used to control pests.

Symposium VI Wednesday, 8:40 **89**

Behavioural changes induced to hosts by Entomophthoralean fungi: mechanisms and evolutionary traits

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Fungi from Entomopthorales are unique insect pathogens: most species are obligate insect pathogens, they can rapidly develop epidemics in host populations, most species discharge conidia actively, and they can survive periods without the host as resistant resting spores. Moreover, they are able to induce behavioural changes in their hosts in several ways. Well known is the so called 'summit disease', observed for example in infected ortoptherans and dipterans. Moribund insects move to the top of vegetation, get fixed there and disperse conidia from this position, for the benefit of the fungus. This behaviour has a diurnal rhythm, so that the host death and following spore dispersal ensure optimal environmental conditions for the fungi – high humidity. However, several other behavioural changes have been documented. Species from the *Entomophthora muscae* complex may induce copulations behaviour and an example of changes in egg laying behaviour is also known. Examples from various host-pathogen systems will be categorized, and we will discus benefits for host or pathogen. Special attention will be given to our newly initiated study on

Formica rufa ants infected with *Pandora formicae*, a rare example of Entomopthhorales infecting a social insect. We have observed several behavioural changes induced by the fungus. The changes influence both how and where the moribund ants place themselves. In this particular system healthy workers perform "hygienic behaviour" they localize and try to pull away fungus killed cadavers, and by that putting themselves at risk. Mechanisms and evolutionary traits of the induced behavioural changes will be discussed.

Symposium VI Wednesday, 9:20 **90**

Walking with insects: Molecular mechanisms behind parasitic manipulation of invertebrate host behaviour

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Parasitic modification of host behaviour is a widely adopted strategy of parasites to enhance their own transmission. The examples of behavioural manipulation are rapidly accumulating, covering a broad spectrum of parasites and hosts. Nevertheless, surprisingly little is known on the underlying causative physiological, neuronal, hormonal or molecular mechanisms.
A typical case of behavioural manipulation is found in insects infected with baculoviruses. Infected caterpillars show enhanced mobility and start climbing to the top of plants or the forest canopy. As a consequence, the virus is spread over a larger area, thereby increasing the chance to infect a new caterpillar. The baculovirus-insect system provides an excellent platform to study parasitic manipulation of insect host behaviour. It allows the comparative analysis between wildtype viruses and single gene knock-out mutants. Viral genes found to modify host behaviour and potential host genes involved in transducing the virus-induced signal into altered host behaviour will be discussed.

Symposium VII Wednesday 8:00-10:00 Nematodes and Bacteria Divisions

Beyond Agriculture: Nematodes and Bacteria Applications in other Science Disciplines.

Symposium VII Wednesday, 8:00 **91**

***Photorhabdus* and *Xenorhabdus*: A drug discovery goldmine**

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We have shown that *Photorhabdus* and *Xenorhabdus* strains produce an astonishing diversity of secondary metabolites, rivalling that of the traditional source of *Streptomyces*. We have used a combination of novel strain isolation, functional genomics and secondary metabolite analysis to mine these genera for high value bioactive molecules. To date we have identified a library of around 600 novel molecules which we are currently testing in a range of bioassays. The application of powerful post genomic techniques including digital transcriptomics and proteomics have not only guided this bio-prospecting initiative, but also informed on the biology of these important bacteria. This includes a better understanding of the evolution of human pathogenicity in the clinical isolates of *P. asymbiotica*. Importantly the large genetic capacity for secondary metabolite synthesis in these genera is not always reflected in the actual chemical diversity production *in vitro*. To address this shortfall we have applied chromosomal recombineering approaches to place secondary metabolite gene clusters under the control of artificial promoters. In addition pathway reconstruction in *E. coli* and chemical synthesis have also facilitated the production and characterisation of these novel molecules. Finally we have been able to correlate the diversity of secondary metabolite production with genetic relatedness to produce a novel chemical-phylogenetic tree approach to characterise these genera. We find that the unparalleled chemical diversity spectra of these genera also correlates with geographic location and we propose that more extensive global sampling will lead to the production of an even larger library of useful and novel drug-like molecules.

Symposium VII Wednesday, 8:30 **92**

Endotoxin plasmids of *Bacillus thuringiensis*: from simple to complex genetic symbionts

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Although widely viewed as a distinct species, *Bacillus thuringiensis*, is more easily understood as a wide variety of *Bacillus cereus* isolates that bear plasmids coding for insecticidal protein endotoxins and other genes that are typically expressed during sporulation. Endotoxin proteins, such as those belonging to the Cry and Cyt classes, and even those that code for the enigmatic parasporin proteins, are almost always encoded on plasmids, rarely by the bacterial chromosome. As such, these plasmids are genetic symbionts that provide *B. cereus* with a selective advantage when spores bearing these plasmids are consumed together with endotoxin proteins, typically in the form of parasporal crystals, by an appropriate insect host. In the case of plasmids that encode Cry1 proteins, this would be a lepidopteran host, whereas in the case of coleopteran hosts, the plasmids would have to have encoded Cry3 or certain Cry1 proteins, and for mosquito (dipteran) larvae, typically combinations of Cry4, Cry11 and Cyt proteins. Studies of the plasmids that code for Cry and Cyt proteins, especially the former, show that these typically range in complexity from being relatively simple (50 - 75 kb), coding for only a single endotoxin, on up to complex plasmids (125 – 225 kb) coding for multiple toxins and genes coding for factors that regulate the synthesis, crystallization, and packaging of these proteins to optimize host target spectrum and insecticidal efficacy. The focus of most research on endotoxin proteins over the past decade has been on studies of their mode of action and finding new proteins with novel and improved target spectra. In this presentation, I will focus on alternative recent studies on emerging genetic mechanisms encoded by these plasmids that effect trafficking and packaging of complex endotoxin parasporal bodies, studies which are contributing to our understanding of insecticidal bacteria and how to improve their efficacy.

Symposium VII Wednesday, 9:30 **93**

Using nematodes to teach behavior: do worms and zebras really do the same things?

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Pathogens of invertebrates are extremely useful teaching tools for undergraduate classes. Most are small and easy to maintain in laboratories, which makes them attractive from a logistical point of view. Many also have well-known life histories which lend themselves to use as models in teaching laboratories. Teaching animal behavior is a challenging task. First comes choosing which animals to use when illustrating various main concepts of the study of animal behavior. An upper level class on the behavioral ecology of insects presents an interesting problem; it is difficult to convince undergraduate students that

the similarities among animal groups are more compelling than their differences. In other words, the challenge lies in convincing them that worms and zebras really do have the same kinds of behaviors. Doing this is especially useful when a laboratory is included in the class; more worms than zebras fit into the standard teaching laboratory. I have used nematodes as teaching tools in two ways. First, I have developed an undergraduate laboratory exercise that can be used in classes on behavior, ecology or parasitology. Second, in a major that requires a senior capstone research project, I have encouraged undergraduate students to use nematodes as the subject of their research. Research projects have varied significantly in subject areas and quality, but the students do learn about parasites and hosts, infection and pathology, and the economic impact of their subjects in addition to testing fundamental hypotheses about ecology and behavior.

Symposium VII Wednesday, 10:00 **94**

Entomopathogenic nematodes in the undergrad biology classroom: lessons in critical thinking

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Critical thinking is increasingly becoming an explicit focus of undergraduate education. While the term means many things to many people, commonly identified elements of critical thinking include development and analysis of arguments, hypothesis testing, data analysis, creative thinking, and problem solving. While the EPN-bacteria system is incredibly complex and offers a range of novel avenues for inquiry, the fundamentals are straightforward, and translate well to exercises designed to promote critical thinking. The basic biology of the system is amenable to semester-long investigation of concepts such as ecology, virulence, foraging behavior, and interspecific competition. This presentation will discuss experiences using the EPN system at the undergraduate level, focusing on student-derived research questions used in freshman biology sections and in mentored undergraduate research projects at Ferrum College and the University of California, Davis. These techniques have been implemented across a range of courses, and the model is effective at communicating basic biology, providing an example of symbiosis and insect pathology, while also promoting highly effective strategies for promoting critical thinking. The presentation will explore common themes across the different settings, identify contrasts in experience when they occur, and both seek and suggest avenues for future integration of nematodes and their symbiotic bacteria into undergraduate education.

Contributed Papers Wednesday, 10:30 -12:15

Bacteria 3

Contributed Papers Bacteria 3 Wednesday, 10:30 **95**

Diversity and potential genomics mobility of the genetic determinants of cereulide, the *Bacillus cereus* emetic toxin

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Bacillus cereus has been associated with two distinct clinical types of food toxi-infection: diarrhea and emesis. Whereas different heat-labile enterotoxins have been suggested to contribute to the diarrheal symptoms, cereulide, a heat-stable toxin has now been confirmed to provoke emesis. In rare cases however, this toxin can also lead to human death. Cereulide is a cyclic dodecadepsipeptide ionophore, produced via non-ribosomal peptide synthesis (NRPS). Early studies had shown that the genetic determinants of cereulide (a 24-kb gene cluster of *cesHPTABCD*) are located on a 270-kb plasmid related to the *Bacillus anthracis* virulence plasmid pXO1, and the *B. cereus* cereulide-producing strains formed a homogeneous group. However, a recent study identified a distinct cereulide-producing group identified as psychrotolerant *B. weihenstephanensis*. Moreover, the location of the cereulide genetic determinants was shown to vary, strongly suggesting genomic mobility of the NRPS cluster. The interior and adjacent DNA sequence of the *ces* gene cluster from eight cereulide-producing strains, representing different types, were sequenced and analyzed. Sequence variation depending on different cereulide-producing group was noticed. The most striking observation was the identification of two copies of insertion elements (named ISBwe2) with a perfect 16 bp inverted repeat (IR) flanking the up- and down- stream of the *ces* gene cluster of two psychrotolerant *B. weihenstephanensis* strains, indicating the transposition origin. Although no obvious transposon-related sequence was found on other cereulide-producing strains, special secondary structures formed by palindromic repeats and domains related with DNA cleavage and rejoining were identified, indicating recombination trace. Moreover, the conjugation experiments were performed to survey the potential horizontal transfer capability of the cereulide-producing plasmids, which indicated that these plasmids are not self-transmissable or mobilizable. However, the cereulide-producing *B. cereus* group strain can be the potential host for conjugative plasmid pXO16 from *B. thuringiensis*, and the resulting transconjugant has the capability for the retrotransfer of pXO16 to other recipients.

Contributed Papers Bacteria 3 Wednesday, 10:45 **96**

The 54-kDa protein of *Bacillus thuringiensis* subsp. *israelensis* required for parasporal body stability binds to individual endotoxin inclusions during their development

Mercedes Diaz-Mendoza[1], Dennis K. Bideshi[1,2] and Brian A.Federici[1]

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Mosquitocidal strains of *Bacillus thuringiensis* such as *B. thuringiensis* subsp. *israelensis* (ONR-60A) and *B. thuringiensis* subsp. *morrisoni* (PG14) produce multiple crystalline inclusions each bound by multilamellar fibrous matrix (MFM), which also binds the inclusions together to form the mature parasporal body (PB). The individual protoxin inclusions, most notably Cry4Aa, Cry4Ba, Cry11Aa and Cyt1Aa, are encoded by a large plasmid, pBtoxis. Little is known about the composition of the MFM or proteins that are involved in its synthesis and structural stability. Using a proteomic approach we identified five proteins encoded by pBtoxis associated with the mature PB. We demonstrated recently that one of these proteins, Bt152, a novel lectin, is a component of the PB matrix and is required for its stability. In the present study, we used stimulated emission depletion (STED) microscopy to resolve the cellular localization of a Bt152-GFP chimera and track it as the PB developed. Bt152-GFP was found bound to a small locus on each developing crystalline inclusion. The inclusions progressively aggregated to form the mature PB by which time the four discrete Bt152-GFP loci had coalesced to form a single locus on the PB. As previously we showed that Bt152 binds to the PB fibrous matrix and is not found in other structural or soluble component of the cell, our current results suggest that this protein binds to a specific location on the fibrous matrix of individual inclusions as they develop and is likely involved with other proteins, perhaps one or more of the five noted above, that assist in the aggregation of these inclusions to form the mature PB.

Contributed Papers Bacteria 3 Wednesday, 11:00 **97**

New mechanisms for "host iron" acquisition in *Bacillus cereus and B. thuringiensis*

Diego Segond[1], Elise Abi khalil[1], Christophe Buisson[1], Fadi Bou Abdallah[2], Mireille Kallassy[3], Didier Lereclus[1] and Christina Nielsen-LeRoux[1]

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The ability of *B. cereus* and B*. thuringiensis* to colonize various mammals and insects is linked to the presence of several adaptation factors, one of which is the capacity to acquire iron. Previously, an *in vivo* screen of *B. cereus* led to the identification of a novel protein, IlsA, which is specifically expressed in the insect hemocoel and under iron restrictive conditions *in vitro*. It was further shown that IlsA is localized on the surface of *B. cereus* and affinity tests revealed that IlsA interacts with both hemoglobin and host ferritin. Inactivation of *ilsA* decreases the ability of *B. cereus* to grow in the presence of especially ferritin indicating that IlsA plays a role in iron acquisition from this iron source. In addition, the *ilsA* mutant displays reduction in growth and virulence in an insect model *Galleria mellonella* (PloS Pathogens, 2009 (11) e1000675). In order to further analyze how IlsA takes part in iron acquisition ferritin we are actually searching for possible partners playing a role in transport and iron release. To understand how iron is released from host ferritin and transported into the bacterial cells, investigations on the interaction between IlsA and ferritins and on the roles of the *B. cereus* siderophores (bacillibactin and petrobactin) have been done. Our data suggest that IlsA may contribute to unfold the ferritin shell. This finding reveals for the first time the mechanisms of host ferritin use by bacteria and highlights the interplay between surface proteins and siderophores, which might be a common mechanism in both vertebrates and invertebrates.

Contributed Papers Bacteria 3 Wednesday, 11:15 **98**

Interactions of five Cry toxins with larval midgut binding sites of *Ostrinia furnacalis* (Guenée)

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Pyramiding or stacking multi-genes is one of many proposed strategies to manage target pests resistance to *Bt* crops. However, a critical requirement for the multi-genes strategy to work is that pyramided or stacked toxins bind to different receptors. The bioassay data with Cry1Ab, Cry1Ac, and Cry1F proteins against offspring of the reciprocal crosses between ACB-AbR (a Cry1Ab resistant strain) and ACB-AcR (a Cry1Ac resistant strain) of *Ostrinia furnacalis* (Guenée) showed as resistant, or more resistant, than their homozygous resistant parents and suggested that resistance and cross-resistance alleles were complementary. In contrast, either two resistant strains or the offspring of their reciprocal crosses were equally susceptible to Cry1Ie as the susceptible strain. The binding of biotinylated Cry1Ab, Cry1Ac, Cry1Ah, Cry1F, and Cry1Ie to brush border membrane vesicles (BBMVs) of *O. furnacalis* was analyzed in competition binding assays with each other of unlabelled toxins as homologous or heterologous competitors, respectively. Homologous competition assays showed that those toxins bind with high affinity to binding sites on BBMVs of *O. furnacalis*. Heterologous competition assays demonstrated that Cry1Ab, Cry1Ac, and Cry1Ah competed for common binding sites. Cry1F competed partially with Cry1Ab, Cry1Ac and Cry1Ah. However, Cry1Ie did not compete to any of the other four toxins bindings. These results indicate that Cry1Ab, Cry1Ac, and Cry1Ah binding sites are shared each other on the BBMVs of *O. furnacalis*, and are partially shared by Cry1F, but Cry1Ie binding sites are not recognized by Cry1Ab, Cry1Ac, Cry1Ah, and Cry1F.

Contributed Papers Bacteria 3 Wednesday, 11:30 **99**

***Bacillus thuringiensis* Cry34Ab1/Cry35Ab1 binding sites on *Diabrotica virgifera virgifera* LeConte midgut membranes are distinct from binding sites for Cry3Aa, Cry3Ba, Cry6Aa and Cry8Ba**

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Bacillus thuringiensis Cry34Ab1/Cry35Ab1 are structurally unique binary insecticidal proteins active against the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. When co-expressed as insect resistance traits in maize, these Cry proteins are an important tool for corn rootworm management. Maize varieties protected by Cry34Ab1/Cry35Ab1 (event DAS-59122-7) have been commercialized by Dow AgroSciences under the trade names Herculex™ RW and Herculex Xtra®. With the aim to protect the long term durability of Cry34Ab1/Cry35Ab1 trait technology we investigated the compatibility of these binary proteins in combination with other coleopteran-active Cry proteins as pyramids for insect resistance management. Binding site interactions were investigated between Cry34Ab1/Cry35Ab1 and Cry3Aa, Cry3Ba, Cry6Aa, and Cry8Ba on western corn rootworm midgut brush border membrane vesicles (BBMV). Displacement of **^{125}I-** radiolabeled proteins bound to BBMV was used as a measure of shared binding sites. Our work demonstrated that ^{125}I-Cry35Ab1 binds specifically to rootworm BBMV. Two primary lines of evidence presented here support the lack of shared binding sites between Cry34Ab1/Cry35Ab1 and the aforementioned proteins: 1) No competitive binding to rootworm BBMV was observed for unlabeled Cry34Ab1 and Cry35Ab1, or a combination of the two, when used in excess with any of the labeled proteins ^{125}I-Cry3Aa, ^{125}I-Cry3Ba, and ^{125}I-Cry8Ba, and 2) Bound ^{125}I-Cry35Ab1 combined with unlabeled Cry34Ab1 was not displaced by any of the unlabeled competitor proteins. These findings provide evidence that Cry34Ab1/Cry35Ab1 are likely to be compatible with insect resistance traits based on Cry3Aa, Cry3Ba, Cry6Aa, or Cry8Ba for deployment as insect resistance management pyramids for in-plant control of western corn rootworm.

Contributed Papers Bacteria 3 Wednesday, 11:45 **100**

Aminopeptidases function as Cry11A toxin binding proteins in *Aedes aegypti*

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The Cry11Aa protein produced in *Bacillus thuringiensis* subsp. *israelensis,* a bacterial strain used worldwide for the control of *Ae. aegypti* larvae, is one of the more toxic proteins to this mosquito. The Cry11Aa toxin binds *Ae. aegypti* brush border membrane vesicles (BBMV) with an apparent K_d of 28.86 nM. Previously an aminopeptidase N (APN), named AaeAPN2, was identified as a putative Cry11Aa toxin binding protein by pull-down assays using biotinylated Cry11Aa toxin (Chen et al., (2009) Insect Biochem Mol Biol., 39: 688-696). Here we show this protein localizes to the apical membrane of epithelial cells in proximal and distal regions of larval caeca. The AaeAPN2 protein binds Cry11Aa with high affinity, 8.6 nM. The full-length and fragments of AaeAPN2 were cloned and expressed in *Escherichia coli.* The toxin-binding region was identified and further competitive assays demonstrated that Cry11Aa binding to BBMV was efficiently competed by the full-length AaeAPN2

and the fragments of AaeAPN2b and AaeAPN2e. In bioassays against *Ae. aegypti* larvae, the presence of full-length and a partial fragment (AaeAPN2b) of AaeAPN2 enhanced Cry11Aa larval mortality. Taken together, we conclude that AaeAPN2 is a binding protein and a functional receptor for Cry11Aa toxin.

Contributed Papers Bacteria 3 Wednesday, 12:00 **101**

Cyt1A of *Bacillus thuringiensis* subsp. *israelensis* forms small aggregates on the midgut epithelium cell membrane of *Culex quinquefasciatus* larvae

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Two hypotheses on the mode of action of Cyt1A have been proposed. One postulates this toxin self-assembles into hexamers that form cation-selective channels that result in colloid-osmotic lysis. The second hypothesis suggests that Cyt1A aggregates non-specifically on the membrane surface and acts in a detergent-like manner. Here we addressed the issue of the oligomeric state of Cyt1A within the plasma membrane of midgut cells using Total Internal Reflection Fluorescence (TIRF) microscopy combined with the Number and Brightness (N&B) method of analysis which measures the average aggregation of Cyt1A. *Culex quinquefasciatus* larvae were exposed to fluorescently labeled Cyt1A toxin. The midgut was dissected and cells were gently teased apart. Cells were imaged using a TIRF microscope, exciting at 488 nm. 2000 images were collected at 512 x 256 pixels with 50,000 microseconds exposure time. N&B analysis was then performed to obtain the aggregation state of the toxin at each pixel. From this analysis we constructed maps of intensity, molecular brightness and the number of Cyt1A molecules. All analysis as performed using the software SimFCS (Laboratory for Fluorescence Dynamics, Irvine, CA). Based on TIRF/N&B analysis, we demonstrated the presence of Cyt1A dimers in the plasma membrane. These results rule out the cation channel hypothesis, since we did not detect higher aggregates at concentrations known to cause microvillar membrane lesions *in vivo*. Our results also differ from the hypothesis of the detergent-like mechanism suggesting a different Cyt1A mode of action.

Contributed Papers Wednesday, 10:30 – 11:30

Diseases of Beneficial Invertebrates 1

Contributed Papers DBI 1 Wednesday, 10:30 **102**

Presence of the Israeli acute paralysis virus in honey bee collapsing colonies

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The Colony Collapse Disorder (CCD) is a special case of collapse of honeybee colonies that has resulted in sudden losses for beekeepers in USA, Europe, Israel, China and Australia. CCD-affected colonies lose most of the adult bee population and are left with a live queen, abundant brood, plenty of food and no dead honey bees in the hive. The Israeli acute paralysis virus (IAPV) was associated with CCD. RNA interference (RNAi) efficiently inhibits replication of RNA viruses by detecting dsRNA intermediates formed during their replication. We performed analysis of the small RNA populations present in CCD and IAPV-infected honey bee colonies using deep sequencing to study the impact of the viral infection. Analysis of the data obtained points out several characteristics of the response of the host to the viral infection that will be discussed.

Contributed Papers DBI 1 Wednesday, 10:45 **103 STU**

CBP, a new member of CBM33 family, is an important virulence factor of *Paenibacillus larvae*, the causative agent of AFB

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American foulbrood (AFB) is considered the most contagious and destructive infectious disease in honeybees, caused by the Gram-positive, spore-forming bacterium *Paenibacillus larvae*. Despite the unquestionable impact of this disease, molecular mechanisms involved in pathogenesis still remain elusive. *P. larvae* spores proliferate massively in the midgut of young bee larvae and finally breach the epithelium. However, to achieve their way through the gut, bacteria must first penetrate the peritrophic matrix, a chitin-rich protective layer of the larval gut. Therefore, we hypothesized that chitin, the major component of the peritrophic matrix, may be target for pathogens. Here, we present our data on a chitin-bindin protein (CBP) secreted by *P. larvae*. Although this protein family has been traditionally described as non-catalytic it was recently shown that CBP21 from *Serratia marcescens* is an oxidative enzyme boosting the enzymatic breakdown of chitin by a different method than chitinases. We combined transcriptomic, proteomic and histological studies, both in vivo and in vitro, to functionally characterize CBP of *P. larvae*. We demonstrate that CBP acts similarly to CBP21 and that it indeed plays an important role during *P. larvae* infection. This is the third catalytic CBP characterized and the first one related to pathogenesis.

Contributed Papers DBI 1 Wednesday, 11:00 **104**

***Nosema ceranae* rebounds from fumagillin control**

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Fumagillin is the only approved antibiotic to control nosema disease in honey bees and has been extensively used in United States apiculture for more than 50 years. It is known to be toxic to mammals and must be applied periodically and with caution to avoid residues in honey. We show that the current application protocol for fumagillin may exacerbate microsporidia infection rather than suppress it, allowing hyperproliferation of the pathogens, particularly *Nosema ceranae*, when the drug is naturally degraded or diluted to low levels as occurs in hives over the spring and summer. Further investigations suggest that fumagillin continues to alter proteins in the honey bee midgut under very low dosages. *N. ceranae* is apparently released from the suppressive effects of fumagillin at dosages that continue to impact the bee midgut tissues, resulting in spore production that is significantly higher than in untreated bees.

Contributed Papers DBI 1 Wednesday, 11:15 **105**

Nitric Oxide participation in *Apis mellifera* hemocytic immune activation upon recognition of non-self and encapsulation

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The honey bee *Apis mellifera* (Hymenoptera) is affected by many parasitosis representing a serious threat to our ecosystem and apiculture. Studing *A. mellifera* immune system contributes with rewarding information for developing new strategies to confront honey bee diseases. Elimination of organisms into the insect hemocoel requires that blood cells (hemocytes) recognize and respond to the invader. Up to date the data concerning cellular immune responses of *A. mellifera* is scarce. After recognition of non-self, hemocytes 'spread'. If the foreign agent is small, this spreading promotes phagocytosis, whereas a larger object triggers an encapsulation response. Hemocyte spreading in response to biotic (bacterial elicitors) and abiotic (plastic or glass) surfaces is measure of immune activation. Nitric oxide (NO) is a signaling and immune effector molecule synthesized by the enzyme nitric oxide synthase (NOS). In insects, NO production has been reported in response to microbial infection in several species of lepidopterans, hemipterans, and dipterans. In this work we studied the participation of NO in the hemocytic responses of *A. mellifera* upon recognition of non-self and encapsulation in-vitro. Experiments were conducted over primary cultures of *A. mellifera* spinning larvae and one-day-old workers hemocytes. Flagellin treatments enhanced *A. mellifera* hemocytes spreading and NO production in an L-arginine dependent process. Glass adherent hemocytes produced great amounts of NO. When treated with the NO scavenger cPTIO, hemocyte spreading over glass surfaces was reduced. Our results suggest that NO participates in *A. mellifera* immune response to non-self and at the beginning of an encapsulation response in honey bees.

Contributed Papers Wednesday, 10:30 – 12:00

Microbial Control 2

Contributed Papers Microbial Control 2 Wednesday, 10:30 **106**

Can *Beauveria bassiana* be a part of strawberry IPM in California Central Coast?

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California Central Coast is the largest strawberry growing region in the US producing nearly 90% of the strawberries for fresh and processing markets. Lygus bug, twospotted spider mite, thrips, and occasionally whiteflies are important pests of strawberries causing significant damage to the quality and yield. *Beauveria bassiana* is pathogenic to most of the strawberry pests and a few commercial formulations are registered for both conventional and organic strawberries. With mild temperatures, nighttime condensation on the foliage, foggy conditions in parts of spring and early summer, and a good amount of rainfall, coastal weather is ideal for microbial control agents such as *B. bassiana*. Preliminary laboratory, greenhouse, and field studies demonstrated the potential of *B. bassiana* to be a part of strawberry IPM and helped improve the awareness of microbial control in the strawberry grower community. Additional field studies are underway to evaluate *B. bassiana* in conventional strawberries.

Contributed Papers Microbial Control 2 Wednesday, 10:45 **107**

Compatibility of fruit fly attractants with *Metarhizium anisopliae* for the management of *Bactrocera invadens*, an invasive pest of horticulture in Africa

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The invasive fruit fly, *Bactrocera invadens* is one of the most devastating pest of fruits and vegetables in Africa. The entomopathogenic fungus, *Metarhizium anisopliae* isolate ICIPE 69 has been identified to be highly pathogenic to adult *B. invadens*. However, application of the fungus requires integration into baiting technique; the conventional management strategies for fruit flies. We tested the compatibility of *M. anisopliae* ICIPE 69 with several male and female attractants in the laboratory and field cages for *B. invadens* management. Direct mixture of varying concentrations of liquid methyl eugenol (ME) with aqueous suspension of conidia was detrimental to the fungus. However volatiles emanating from cotton wick and polymeric plugs of ME had less effect on conidial germination (85 and 92%, respectively). Mortality of *B. invadens* visiting ME bait stations encompassing dry conidia of *M. anisopliae* was 92% at 5 d post-exposure. Four liquid female biased protein baits (NuLure, Mazoferm, HymLure, DuduLure) were compatible with the fungus (86-94% germination). However, mortality of *B. invadens* visiting sprayed suspension of protein bait and *M. anisopliae* was low (32-48% at 5 d, post-exposure), suggesting poor uptake of spores from treated surface. Flies arriving at dry protein bait stations treated with dry conidia of *M. anisopliae* incurred significantly higher mortality (87-92% at 5 d, post-exposure) compared with the liquid bait (35%). The fungus persisted for up to 21 days on dry bait stations. The significance of these finding in the management of *B. invadens* on mango orchard is discussed.

Contributed Papers - Microbial Control 2 Wednesday, 11:00 **108**

Use of *Metarhizium anisopliae* to control the leafhopper: characterization of two major commercial production areas of sugar cane in Brazil

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The use of *Metarhizium anisopliae* for sugarcane leafhoppers control has grown fastly in Brazil. The climatic conditions and the great diversity favored epizootic insect diseases. It makes Brazil one of the largest producers of biological control agents for sugarcane. The use of this fungus began in the Northeast of the country and is growing in the Southeast due to efficiency, low cost and minimal environmental impact. Despite the Southeast is a major producer of sugarcane and makes use of biological control on a large scale, there is few information about biofactories. This paper discusses an overview of the production and marketing of *M. anisopliae* in two sugarcane producers cities in São Paulo (Southeastern Brazil): Araçatuba and Sao Jose do Rio Preto. Sugarcane farmers were also interviewed. It was observed that there is not biofactories in S. J. Rio Preto city, but in some neighboring cities (those was interviewed). In the Araçatuba city there are biofactories linked or not to sugarcane industry. In all the companies interviewed the production system is handcrafted, using rice as substrate. The biofactories generally provide training and follow-up during biological control application. The sugarcane producer that provides for the industry does not use biological control because the lack of information and product. The demand by the fungus is greater than the supply market. This work will provide subsidy to researchers and companies in the area, to the farmer who wishes to use this product in fields and as a means of spreading this biological control strategy.

Contributed Papers - Microbial Control 2 Wednesday, 11:15 **109**

Research on Metarhizium for Wireworm Management – Retrospective and Foresight

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Ten years of research have advanced our understanding of using Metarhizium for wireworm control. Heightened strain virulence does not seem to be enough, so the application of the knowledge gained by studying wireworm behaviour and pathogenesis in relation to environmental variables, cropping

practices, and biocontrol applications has focussed Metarhizium use patterns for inundative application. For example, Metarhizium efficacy, even at exceedingly low application rates, has been greatly synergized in causing wireworm mortality when used in combination with other compounds. And while Metarhizium can have a repellent effect on wireworms, it might be overcome by use patterns that make them captive in the zone treated with the fungus. Timing of application is also an important consideration, particularly in temperate zones where cool soils affect both wireworm movement and fungal infection and growth. And are we being too narrow in focus by considering only the larval stage of this Elaterid? We show why adults are also worthy of targeting, with new data from recent field trials.

Contributed Papers - Microbial Control 2 Wednesday, 11:30 **110**

Influence of plant culture conditions on efficacy of foliar applications of entomopathogenic fungi against western flower thrips

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A series of greenhouse tests was conducted to assess the efficacy of foliar applications of two commercially available entomopathogenic fungi, *Beauveria bassiana* strain GHA and *Metarhizium brunneum* strain F52, against western flower thrips infesting potted impatiens grown with subirrigation. Five or six applications were made at 3–4-day intervals using an ESS XT-3™ sprayer and hand-held MaxCharge™ spray gun with the electrostatic generator disconnected (employed simply as a fine mist blower; disk insert 0.4 delivering ca. 2.6 ml/second at 103.4 kPa). Unformulated conidia were applied at a high rate and volume of 2.15 x 10^{14} viable conidia in 4,073 L water/ha. The two pathogens produced similar levels of control. Under conditions of high humidity (RH >75%) maintained for 2 days after each application, thrips populations in pollen-bearing flowers were reduced by 50-65% relative to those in carrier-controls (0.01% Silwet L-77). Substantially greater reductions (75–90%) were recorded in foliage samples. Control was slow to develop, coinciding with plant growth to the point of canopy closure (2–3 weeks after initiation of spray programs). Efficacy was highly dependent upon ambient moisture conditions. Population reductions did not exceed ca. 30% in flowers or 50% in foliage when humidity was low or only intermittently high (e.g., when limited to overnight periods), and control was also significantly reduced, regardless of plant age or humidity conditions, when plots were exposed to ventilation sufficient to produce a constant perturbation (visible movement) of foliage in the crop canopy. These results are in accord with grower reports of inconsistent efficacy of fungal pathogens applied against thrips in greenhouse crops, and indicate that efficacy can be expected to vary markedly with many aspects of crop culture, including ambient humidity, duration and periodicity of high humidity conditions, plant age (size), and plant location relative to air circulation within a greenhouse. Studies are planned to determine the effects of fungal formulation on efficacy under the above-described conditions.

Contributed Papers - Microbial Control 2 Wednesday, 11:45 **111**

Development of *Metarhizium anisopliae* strain F52 in North America and Europe

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The entomopathogenic fungus *Metarhizium anisopliae* strain F52 is currently registered in the U.S., Europe, and Canada. This isolate has potential for application in a wide range integrated pest management programs. This presentation will highlight progress we have made in soil applications against vine weevils and thrips and in foliar applications against thrips, whiteflies, mites, and ticks. Key challenges we face such as ensuring adequate coverage, persistence and integration with other control products will be discussed. Also key opportunities we see such as insect resistance management, expansion to new targets, and colonization of the rhizosphere will also be discussed. Use patterns are currently being refined including application timing, placement, compatibility with beneficials and agrochemicals, and rotation with insecticides. These new products offer an attractive new tool for the integrated pest management and resistance management.

Contributed Papers Wednesday, 10:30 – 11:45

Nematodes 2

Contributed Papers Nematodes 2 Wednesday, 10:30 **112 STU**

***Delia platura,* Meigen 1826 (Diptera: Anthomyiidae) control with entomopathogenic nematode Steinernema sp3 JCL027 in Cota (Cundinamarca), Colombia**

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The seed maggot, *Delia platura*, is the major pest of spinach crops in the sabana Bogota. Therefore, different doses, application time and recovery of *Steinernema* sp3 for *D. platura* control were examined in a field experiment using a commercial spinach (*Spinacia oleracea* L.) crop as the host plant. The crop was located in Alcala (Cota – Cundinamarca). Since germination to harvest, there were three application treatments. The doses inoculated per plant were 500, 1000,2000,4000,8000 infective juvenile (IJ) and chemical control used by the producer. The application of *Steinernema* sp3 during three phonological stages of the crop resulted in a reduction of 50% of the damage at doses of 4000 to 8000 IJ relative to control that showed 67% damage, taking into the damage caused by the plague is greater during germination and harvest. Doses of 500 to 1000 IJ showed a similar damage that registered by absolute and relative control. *Steinernema* sp3, is alternative for seed maggot control in fields and could be involved in spinach crops management strategies.

Contributed Papers Nematodes 2 Wednesday, 10:45 **113**

Insect host diet and its impact on the fitness of entomo-pathogenic nematodes and their symbiotic bacteria

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In this study we considered the tripartite system represented by pathogenic nematodes, their symbiotic bacteria and insect host to assess costs and benefits of the physiological condition of one of the three partners – the host insect –may affect the system as a whole. The tobacco horn worm, *Manduca sexta* (Lepidoptera: Sphingidae), was considered as the insect host. Two entomopathogenic nematode spp.: *Steinernema carpocapsae* ALL strain and *Heterorhabditis sonorensis* Caborca strain, and their cognate bacterial symbionts, *Xenorhabdus nematophila* and *Photorhabdus luminescens*, respectively, were examined to assess the effects of insect host diet on pathogen/symbiont performance. *M. sexta* was reared on artificial diet under "low" and "high" quality conditions. We evaluated the impact of insect diet choices on nematode fitness and bacterial symbiont colonization and proliferation. Insect host mortality, EPN time to emergence, nematode progeny production and number of bacterial cfu/ IJ were assessed. Insect host mortality was generally higher for *M. sexta* reared

on low quality diet relative to those reared on the optimal, high quality diet. In addition, *M. sexta* exposed to *S. carpocapsae* had significantly higher mortality than those exposed to *H. sonorensis*. The EPN progeny production as well as time to emergence did not differ based on insect host diet; however, there were significantly fewer IJs per cadaver for *S. carpocapsae* compared to *H. sonorensis*. Finally, the average number of bacterial symbionts per IJ is not different between treatment groups, but there was more variation within the low diet treatment group.

Contributed Papers Nematodes 2 Wednesday, 11:00 **114**

Contributions of cognate and non-cognate symbionts to nematode host fitness

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Entomopathogenic nematodes of the genus *Steinernema* have a mutualistic relationship with *Xenorhabdus* bacteria. It has been shown that *Steinernema* and *Xenorhabdus* have coevolved, however this relationship has varying degrees of dependence. Each Steinernema spp. is known to associate with a single Xenorhabdus sp. However, certain Xenorhabdus spp. (i.e. *X. bovienii*) can have different *Steinernema* spp. as hosts. *Steinernema*'s ability to associate with a suitable bacterial symbiont is thought to play a key role in their ability to killing and reproduce inside its insect host. However, the specificity of this mutualistic relationship has not yet been fully investigated. In this study, we focused on the specificity of nematode-bacteria interactions, considering three *Steinernema* species: *S. intermedium*, *S. oregonense*, *S. puntauvense,* all of which share the same symbiont species, *X. bovienii.* A series of bioassays were set to assess the level of symbiont reassociation and the impact the association with cognate and non-cognate symbionts had on nematode fitness (i.e. virulence and reproduction). Results of this study will be discuss and presented.

Contributed Papers Nematodes 2 Wednesday, 11:15 **115**

Olfactory response of the mite, *Sancassania polyphyllae*, to cadavers and tissues with and without entomopathogenic nematodes: impact on biological control

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Previous studies showed that various scavengers of insects including ants, cockroaches, crickets, wasps, and birds were deterred from feeding on nematode-killed insects. For the insect scavengers, a chemical compound(s) produced by the mutualistic bacteria of entomopathogenic nematodes was responsible for this deterrent behavior and hence called the Scavenger Deterrent Factor (SDF). However, SDF had no deterrent effect on a mite species, *Sancassania polyphyllae* (Acari: Acaridae) which detected a nematode-killed insect in a soil column and consumed the entire cadaver and developing nematodes. These results indicated that the mites were responding to volatiles from the cadaver. Therefore, we conducted a Y-tube olfactometer study to determine if *S. polyphyllae* females show a preference among volatiles from *Polyphylla fullo* (Coleoptera: Scarabaeidae) larvae or infective juveniles (IJs) of an entomopathogenic nematode, *Steinernema glaseri* (Rhabditida: Steinernematidae). Female mites were subjected to one- or two-choice odor sources from (1) living larvae, (2) intact, non-nematode-killed larvae, (3) nematode-killed larvae, and (4) dissected larval tissues, or (5) *S. feltiae* IJs. The results demonstrated that the female mites perceived volatiles associated with insect cadavers and tissues with and without nematodes but not to living larvae or *S. glaseri* IJs. The data suggest that scavenger mites such as *S. polyphyllae* may have profound negative effects on natural and applied biological control. The potential impact may especially be significant in applied biological control if nematode-killed insects are used for controlling insect pests.

Contributed Papers Nematodes 2 Wednesday, 11:30 **116**

Evolutionary relationships between *Deladenus* nematodes parasitizing northeastern North American *Sirex* species

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The parasitic nematode *Deladenus siricidicola* Kamona strain is a biological control agent of the invasive woodwasp, *Sirex noctilio*. Since the discovery of *S. noctilio* in northeastern North America in 2005, a biological control program involving *D. siricidicola* is under consideration. We assessed phylogenetic relationships between native *Deladenus* spp. in the northeastern United States to predict possible non-target effects if *D. siricidicola* is introduced for *S. noctilio* control. Parasitized *Sirex* spp. were collected inside and outside of the range of *S. noctilio*. DNA was extracted from nematodes, and three genes (CO1 mitochondrial DNA, 28S ribosomal DNA, and ITS ribosomal DNA) were sequenced and analyzed. Results show two major clades, representing the two "superspecies" proposed by Chitambar (1992). Each of the four species of *Sirex* has a corresponding nematode parasite. Within two *Sirex noctilio* we found nematodes that we hypothesize are normally associated with *S. nigricornis*. One individual of the native *Sirex nigricornis* contained *Deladenus* normally found in *S. noctilio*. We discuss nematode-host fidelity in this system and the potential for non-target impacts of a biological control program using *D. siricidicola* against *S. noctilio*.

Contributed Papers Wednesday, 10:30 – 11:45

Viruses 3 *Functional Genomics I*

Contributed Papers Viruses 3 Wednesday, 10:30 **117 STU**

Protein tyrosine phosphatase-induced hyperactivity is an evolutionarily conserved strategy of baculoviruses to manipulate lepidopteran host behavior

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Many parasites manipulate host behavior to increase the probability of transmission. To date, direct evidence for parasitic genes underlying such behavioral manipulations is scarce. Here we show that the baculovirus *Autographa californica* nuclear polyhedrovirus (AcMNPV) induces hyperactive behavior in *Spodoptera exigua* larvae at three days after infection. Furthermore, we identify the viral protein tyrosine phosphatase (*ptp*) gene as a key player in the induction of hyperactivity in larvae, and show that the phosphatase activity of the encoded enzyme is crucial for this behavioral change. Phylogenetic inference points at a lepidopteran origin of the *ptp* gene and shows that this gene is well-conserved in a group of evolutionarily related baculoviruses. Our study suggests that *ptp*-induced behavioral manipulation is an evolutionarily conserved strategy of this group of baculoviruses

to enhance virus transmission, and represents an example of the extended phenotype concept. Overall, these data provide a firm base for a deeper understanding of the mechanisms behind baculovirus-induced insect behavior.

Contributed Papers Viruses 3 Wednesday, 10:45 **118 STU**

SeMNPV genotypic variants with increased replication efficiency in cultured *Spodoptera exigua* cells lack a gene with pro-apoptotic activity

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The *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) isolate SeUS1 is a complex mixture of genotypes. SeUS1 viral DNA was used for the construction of bacmids by direct cloning in *E. coli.* A complete bacmid (SeBac10) and several variants were generated. Variant SeBac72 retained oral infectivity in larvae but had a much higher transfection efficiency in Se301 and SeUCR cells as compared to SeBac10. Moreover, SeBac10 serial passaging in cell culture generated large deletions in the region ORF15-41, whereas SeBac72 remained stable. We hypothesized that one or more gene(s) in the SeBac72 deleted region prevents successful virus replication in cell culture. Genome sequencing of SeBac72 revealed a 9.5 kb deletion spanning ORF16-28. Transient expression in Sf21 cells showed that one of these ORFs induced the formation of apoptotic bodies and upregulated effector caspase activity. These findings make this gene a candidate responsible for the observed lower transfection efficiency of SeBac10. Bacmids with different ORF16-28 knockouts were constructed to assess transfection efficiency and the induction of apoptosis in *S. exigua* cells. These results indicate that baculoviruses, which clearly rely on anti-apoptotic genes for replication, may also express proteins with pro-apoptotic activity in the *in vivo* viral life cycle.

Contributed Papers Viruses 3 Wednesday, 11:00 **119 STU**

Determination of the role *me53*/ME53 plays in both early and late phases in the baculovirus replication cycle

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AcMNPV *me53*, a highly conserved immediate early gene, is found in all sequenced lepidopteran baculoviruses. ME53 contains a C4 zinc finger domain at the C-terminus, whose function is not clear. It translocates to the nucleus and colocalizes at the cell membrane with viral envelope protein GP64 in the late phase during infection. However, what mechanism ME53 uses to localize to the nucleus and whether ME53 interacts with other viral or host proteins to assist this translocation are unknown. A series of truncated ME53-HA bacmids were constructed and immunofluorescence assays were performed to identify which region determines ME53 nuclear localization. The peptide ME53 AA (83-152) was able to translocate into the nucleus, while ME53 with AA (83-152) deleted failed to localize in the nucleus. Meanwhile, although ME53 AA (33-82) can partially localize to the nucleus, the majority remains in the cytoplasm. The same largely cytoplasmic localization was observed when AA (33-82) was deleted. This indicates that ME53 AA (83-152) is required for the nuclear localization, and AA (33-82) may facilitate this process. Since there are two alpha helixes and one beta strand between amino acid 110 and 150, and they may act as domains that interact with other proteins, it is speculated that ME53 AA (110-150) is responsible for the nuclear localization. Further truncations within this region are being constructed to more finely determine the region for nuclear localization. Immunoprecipitation assay is also being performed to detect the proteins that interact with ME53 and possibly facilitate ME53 nuclear translocation during infection.

Contributed Papers Viruses 3 Wednesday, 11:15 **120**

The distribution and phosphorylation of the basic protein P6.9 of *Autographa californica nucleopolyhedrovirus*

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Baculovirus protein of P6.9 is thought to play a role in the condensation of viral genomes to form nucleoprotein within nucleocapsids. The role of P6.9 in nucleoprotein encapsidation is still unclear although this highly basic protein has been found more than 30 years. We raised an antiserum against unphosphorylated peptides from P6.9 to detect the expressed P6.9 without phosphates in these regions. Additionally, an HA-tagged P6.9 recombinant bacmid was constructed to allow the use of anti-HA antibody to detect 'total' expressed protein. Here, it is unexpected to found that the majority of P6.9 was able to co-localize with marginalized host chromatin. This result implies that P6.9 may have unknown function besides packaging viral DNA. Additionally, immunoelectron microscopy found that differences in phosphorylation state of P6.9 lead to differences in localization in virogenic stoma. Most importantly, a novel Western blot system based on acid urea polyacrylamide gel electrophoresis was improved to separate the different charged basic protein. Western blots results found that only unphosphorylated P6.9 was associated with budded virions, however, abundant phosphorylated forms of P6.9 were associated with occlusion-derived virions. This result suggests that the phosphorylation state of P6.9 may probably determine whether nucleocapsids become budded virions or occlusion-derived virions.

Contributed Papers Viruses 3 Wednesday, 11:30 **121 STU**

Acid activation of the budded virus fusion protein F of *Spodoptera exigua* multicapsid nucleopolyhedrovirus

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Baculovirus budded virions use membrane fusion to deliver their nucleocapsid into the host cell cytoplasm. For most baculoviruses this fusion process is mediated by the F protein. The F protein is proteolytically primed for fusion during protein biogenesis by intracellular-furin cleavage of the precursor F0 into two disulfide-bonded subunits F1 and F2. This results in exposure of a hydrophobic fusion peptide at the N-terminus of F1. The fusion process is activated by low pH, which the virus encounters in endosomes during cell entry. We have expressed and purified the soluble ectodomain of the SeMNPV F protein (F-e) in human embryonic kidney cells and biochemically studied the different states of the cleaved and uncleaved form at low and neutral pH. Native polyacrylamide gel electrophoresis shows that upon cleavage F-e undergoes a conformational rearrangement. The uncleaved F-e does not change in response to a low pH, whereas the cleaved F-e shows different conformations at pH5 and pH7. The acid-induced transitions of F-e likely resemble conformational changes occurring during the viral fusion process. Furthermore, the F protein appeared to be activated at pH5-5.5, which is below the pKa value of histidines. Alignment of SeMNPV F with homologues of other baculoviruses shows the presence of six highly conserved histidine residues. Diethylpyrocarbonate (DEPC), known to modify histidines preventing their protonation, strongly inhibited infection in a dose dependent manner. Mutagenesis of specific conserved histidines significantly effected entry of F-pseudotyped viruses indicating that histidine in F play a role as a pH sensor to initiate viral fusion.

Contributed Papers Viruses 3 Wednesday, 11:45 **122**

Structure-based functional models of fusion peptide of baculovirus envelope fusion protein F

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The N-terminal fusion peptide of membrane anchoring subunit of baculovirus envelope fusion protein F plays a crucial role in membrane fusion and virus entry. We previously reported an amphiphilic "coil$^{1-5}$-helix$^{6-8}$-turn^{9}-helix$^{10-19}$" structure of the fusion peptide of *Helicoverpa armigera* nucleopolyhedrovirus (NPV) F protein (HaF). In this research, we extensively analyzed the structure-function relationship, particularly proposed functional models of fusion peptides of baculovirus F proteins. The structures of four selected HaF fusion peptide mutants N^1G, I^2N, G^3L, $D^{11}L$ and the native fusion peptide of *Lymantria dispar* NPV F protein (LdF) were resolved by NMR technology, and their respective functions were analyzed *in vitro*. The lethal mutation $D^{11}L$, located in the hydrophobic side of the core α-helix$^{10-19}$ motif, completely abolishes the amphiphilic helix and exhibits a random coil structure. The lethal mutation I^2N, although maintaining amphipathicity, is composed of a short central α-helix motif surrounded by extended N-/C-terminal coils. The G^3L mutation exhibits an enhanced helix structure in the N-terminus, but is of substantially decreased fusion activity. The N^1G is the only mutation which increases fusogenicity and its conformation has been changed to a continuous, rigid helix without distinct kink motif as found in the wide type HaF fusion peptide. Interestingly, the fusion peptide of LdF adopts a strikingly similar structure as N^1G. Since class I viral fusion peptide generally contains a kink motif to achieve angled "V" for efficient fusion, this straight helix may represent a novel type of structure for viral fusion peptide. The lipid-inserted residues and the orientations of individual peptide are further analyzed and the results suggest that there is no direct correlation between the insertion depths and the fusion activities. Thus, we propose that both "V" shape and straight helical stick are functional conformations for baculovirus F protein fusion peptide, but they may adopt different mechanisms and functional models in mediating efficient virus-cell fusion.

Contributed Papers Viruses 3 Wednesday, 12:00 **123**

Incorporation of GP64 into Helicoverpa armigera NPV enhances virus infectivity both *in vivo* and *in vitro*

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The envelope fusion protein of baculovirus, which is responsible for viral entry into host cells, is important for viral infectivity. There are two kinds of envelope fusion proteins in baculoviruses: GP64 from group I nucleopolyhedrovirus (NPV) and F protein from group II NPV and Granulovirus (GV). F protein is considered as the ancestral baculovirus envelope fusion protein, while GP64 is relative recently evolved and exhibits more effective fusogenic activity than F protein. In this study, a recombinant virus vHaBac-*gp64-egfp* was constructed from *Helicoverpa armigera* NPV, a group II NPV, which expressed both GP64 from *Autographa californica* nucleopolyhedrovirus (AcMNPV) and its original F protein. The properties of this recombinant virus were investigated *in vitro* and *in vivo*. By one-step growth curve analysis and Q-PCR determination of viral DNA copies, the recombinant virus vHaBac-*gp64-egfp* was showed more infectious than the control virus vHaBac-*egfp in vitro*. When *polyhedrin* gene (*polh*) was re-introduced into the recombinant viruses, bioassay analyses showed that vHaBac-*gp64-polh* accelerated the killing of the infected insect larvae in comparison to the control virus vHaBac-*egfp-polh*. The median lethal concentration (LC_{50}) of vHaBac-*gp64-polh* reduced about 80% of that of vHaBac-*egfp-polh*, and the insertion of GP64 significantly shorten the median survival time (ST_{50}). In summary, incorporation of GP64 into HearNPV BVs improved virus infectivity both *in vivo* and *in vitro*. The recombinant virus containing bivalent fusion proteins could be developed as more effective bio-pesticide.

Symposium VIII Wednesday, 14:45 - 16:30
Bacteria Division

Bacterial topics of interest to Latin America

Symposium VIII Wednesday, 14:45 **124**

Assessment of the high-dose concept and level of control provided by MON 87701 × MON 89788 soybean in Brazil

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Genetically modified MON 87701 × MON 89788 soybean (*Glycine max*), which expresses the Cry1Ac and EPSP-synthase proteins, has been registered for commercial use in Brazil. To develop an Insect Resistance Management (IRM) program for this event, laboratory and field studies were conducted to assess the high-dose concept and level of control it provides against *Anticarsia gemmatalis, Pseudoplusia includens and Heliothis virescens.* The purified Cry1Ac protein was more active against *A. gemmatalis* [LC50 (FL 95%) = 0.23 (0.15–0.34) µg Cry1Ac mL^{-1} diet] than *P. includens* [LC50 (FL 95%) = 3.72 (2.65–4.86) µg Cry1Ac mL^{-1} diet]. The Cry1Ac purified protein incorporated into artificial diet, showed that Cry1Ac was highly biological active against *H. virescens* [LC50 (95% FL) = 0.026 (0.021 - 0.033) µg Cry1Ac mL^{-1} diet]. In bioassays with freeze-dried MON 87701 × MON 89788 soybean tissue diluted 25 times in an artificial diet, there was 100% mortality of *A. gemmatalis and H. virescens* and up to 95.79% mortality for *P. includens*. In leaf-disc bioassays and under conditions of high artificial infestation in the greenhouse and natural infestation in the field MON 87701 × MON 89788 soybean showed a high level of efficacy against *A. gemmatalis, P. includens and H. virescens*, but a complete high-dose event only to *A. gemmatalis* and *H. virescens*

Symposium VIII Wednesday, 15:10 **125**

Vip3A, a novel mode of insecticide action to improve productivity and sustainability

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In Latin-American, the main pests for corn are from the Lepidoptera order; being *Diatraea saccharalis* (Sugarcane Borer), *Spodoptera frugiperda* (Fall Armyworm) and *Helicoverpa zea* (Corn earworm) the main species. This are very difficult to be controlled by insecticides because of their sudden and severe attacks; combined with their growing habits inside the plant, therefore they cause significant economical loses. The first GM "Bt" plants, expressing a Cry1ab protein, solved very well the problems caused by the Sugarcane borer, leading to direct and indirect benefits for the growers; but limited control was observed for Fall Armyworm and Corn Earworm. For several years Cry1Ab was the main insecticidal protein in the field in Argentina, being the only selection pressure for resistance breaking biotypes in *Diatraea*. Since the commercial approval of Agrisure Viptera, a completely new mode of insecticidal action is in the field, helping IRM management and improving the sustainability of the production system. In

Argentina, Vip3A showed a total control of the three main Lepidoptera pest as no other available commercial proteins, improving significantly the yield of the hybrids under pest pressure. Also, the high level of control of Corn earworm reduces even more the level of micotoxins in "Bt corn", since the entrance to the ear and the grain caused by the insect damage was reduced to almost cero. Therefore, Vip3a in Argentina means higher sustainability for the Bt corn technology, higher yields and quality, with less insecticide applications.

Symposium VIII Wednesday, 15:35 **126**

Systemic utilization of *Bacillus thuringiensis* – a new tool for pest control

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Bacillus thuringiensis is a bacterium that has been used in biological control for many years, however, although many products are available, their use is still fairly limited. Among the limitations of their use may be cited two in particular – low persistence in the field of formulations due to toxin degradation by sunlight and difficulty of some sucking and endophytic insects and nematodes in acquiring the toxin available in biopesticides. Recently, studies are being conducted to verify the possibility of using strains of *B. thuringiensis* in a systemic way. It was demonstrated that some strains, once inoculated into the roots of some plants, are absorved by them and can be retrieved in the guts of insects that fed on it. These studies are opening up the possibility of a new strategy for controlling insects and nematodes, thus circulating in the plant, these toxins will be available for sucking and nematodes. Furthermore, these toxins will not suffer the action of sunlight and will probably have a higher persistence in the field**.**

Symposium VIII Wednesday, 15:55 **127**

***Bacillus thuringiensis* crystal proteins as cures for intestinal roundworms**

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Intestinal roundworms (hookworms, whipworms, *Ascaris*, *Strongyloides*) infect somewhere between 1-2 billion people in the world and are prevalent in Latin and South America. A recent survey in the Amazon, for example, found 25% infection rates with *Ascaris* and 25% infection rates with whipworms in children. Intestinal roundworms are considered a top cause of disease burden in children, with infections leading to growth and cognitive stunting, lower future earnings, malnourishment, decreased school attendance, and immune defects. The drugs currently available have significant deficiencies and so new therapeutics are urgently needed.
Our group has pioneered work on *Bacillus thuringiensis* (Bt) crystal (Cry) proteins that target roundworms. Here we will discuss the efficacy of Cry5B against intestinal roundworms. We will present recent data comparing the *in vitro* efficacy of Cry5B versus drugs used clinically against parasitic roundworms. We will present new *in vivo* data on the excellent efficacy of Cry5B in clearing intestinal roundworm infections in small animals when delivered orally. We are in process of mutagenizing the entire Cry5B protein, performing alanine-scanning mutagenesis on each amino acid in the toxin domain. This study is revealing many residues that can be mutated in order to improve toxicity against roundworms and will reveal significant new insights into structure/function relationships in Cry proteins. We are also working on novel delivery systems by which Cry proteins could be used to cure intestinal roundworm infections in humans. Our data reveal that Cry proteins have excellent potential to cure human disease via their effects on roundworms.

Symposium IX Wednesday, 14:45 -16:45
DBI and Microsporidia Divisions

New insights into host-pathogen interaction in the Microsporidia

Symposium IX Wednesday, 14:45 **128**

Investigating the secretome of diverse microsporidia

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The Microsporidia are highly adapted parasitic cells, characterized by a spore stage that is filled with a distinctive and complex infection apparatus, yet they also display traits of extreme reduction. They have small genomes, minimal proteomes, reduced organelles and streamlined biochemical pathways, which makes them a simplified system for studying host-parasite interactions. We are currently using Illumina sequencing to generate new sequence data for microsporidia with interesting interactions with their host. We are using this data combined with proteomics to try to identify the core secretome of microsporidia and to identify microsporidian proteins that are commonly involved in host cell invasion and manipulation. In addition, we are interested in understanding which genes in microsporidia encode proteins that allow survival in particular hosts or particular tissues. We have sequenced the genome of the mitten crab parasite *Hepatospora erocheir* and will compare this to the genomes of the related human parasite *Enterocytozoon bieneusi* and *Enterospora canceri*, which lives exclusively in the nucleus of the host of the edible crab. It is hoped that this will give insight into which element of these parasites' proteomes allow them to infect such diverse hosts and cellular compartments.

Symposium IX Wednesday, 15:15 **129**

Genomic insights into the interactions of the microsporidian parasites *Nosema* and their honey bee hosts

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Honey bees (*Apis mellifera*) play a vital role in our lives by assisting pollination of one-third of the world's food crops. However, honey bees suffer from numerous diseases which have caused dramatic declines in honey bees. Nosemosis, a disease caused by microporidian parasite *Nosema*, is regarded as one of the most prevalent and destructive adult honey bee diseases and has been implicated in worldwide colony declines. *N. ceranae* and *N. apis* are two species of the Nosema that are reported to cause *Nosema* disease in honey bees so far. Using genomic approaches, we conducted study to investigate the biological, molecular and genomic feature of two Nosema species. The molecular and biological studies have yield evidence that *N. ceranae* is the more common and predominant infection of two *Nosema* species in honey bees. Sequencing and annotation of the *Nosema* genomes provide a comprehensive overview of the genetic content, structure, and organization of the parasites and give some interesting insights into the complex biological and molecular interactions between the parasites and honey bee host. The comparative genomic analysis led to the identification of genes that are conserved between *N. apis* and *N. ceranae*, and genes that are unique characteristics of the individual species, thereby providing a list of virulence factors that are associated with virulence of the parasites in honey bees. There genes are potential targets for innovative therapeutics to break down the life cycle of the parasite, thereby improving honey bee health.

Contributed Papers Wednesday, 14:45 – 16:30

Viruses 4 *Insect viruses*

Contributed Papers Viruses 4 Wednesday, 14:45 **130 STU**

Structure, Protein Composition, Morphogenesis and Cytopathology of *Glossina pallidipes* Hytrosavirus

Henry M. Kariithi[1, 2, 3], Jan van Lent[1], Monique M. van Oers[1], Adly M.M. Abd-Alla[2], İkbal Agah İnce[1] and Just M. Vlak[1*]

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The proteins comprising the *Glossina pallidipes* hytrosavirus (GpHV) virions contain functional information required for the virus life cycle. Here, structural components and certain aspects of GpHV morphogenesis were investigated using a combination of electron microscopy and mass spectrometry. Cryo-sections of infected host salivary glands showed that GpHV nucleocapsid and envelope are separated by a jacket of proteinaceous matrix (designated here as tegument). Further the entire surface of mature virions consists of helical outer sub-structures. Nucleocapsids were restricted to the nuclei whereas enveloped virions were located in the cytoplasm. Purified virions were separated into nucleocapsid and envelope fractions by treatment with 1% NP-40. The fractions were separated by 12% SDS-PAGE gel and proteins identified by liquid chromatography coupled to electrospray and tandem mass spectrometry (LC-MS/MS). In total, 45 GpHV structural proteins were identified. Seven of these were assigned to the envelope, 15 to the nucleocapsid and 23 to the tegument. Immunoblotting was used to localize proteins encoded by ORFs 10 and 96 on the virion tegument and ORF1 on the envelope component. In addition to virion proteins, a total of 56 host (cellular) proteins were identified as associated to purified virions. Using proteinase K protection assay, 9 of the cellular proteins were verified to be incorporated into mature virion. This study revealed that GpHV envelopment likely involves the ER-Golgi system which is compatible with the cytoplasmic virion assembly. These data may call for re-evaluation of antiviral strategies to control GpHV infection in tsetse fly rearing facilities for the sterile insect technique.

Contributed Papers Viruses 4 Wednesday, 15:00 **131**

Impact of salivary gland hypertrophy virus infection on the mating success of male *Glossina pallidipes*: consequences for the sterile insect technique

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Many species of tsetse flies are infected by a virus (GpSGHV) that causes salivary gland hypertrophy (SGH). Female *Glossina pallidipes* (Austen) with SGH symptoms (SGH**+**) have reduced fecundity and SGH**+** male *G. pallidipes* are unable to inseminate female flies. Consequently, *G. pallidipes* laboratory colonies with a high prevalence of SGH have been difficult to maintain and have collapsed on several occasions. To assess the potential impact of the release of SGH**+** sterile male *G. pallidipes* on the efficacy of an integrated control programme with a sterile insect technique (SIT) component, we examined the mating efficiency and behaviour of male *G. pallidipes* in field cages in relation to SGH prevalence. The results showed significantly reduced mating frequencies in a field cage setting for a male *G. pallidipes* population with a high prevalence of SGH (83 %) as compared to a male population with a low prevalence of SGH (7 %), i.e. 21 % successful mating pairs as compared to 37%, respectively. Premating period and mating duration did not vary significantly with SGH status. A high percentage (>80%) of females that had mated with SGH**+** males had empty spermathecae. The remating frequency of female *G. pallidipes* was very low irrespective of the SGH status of the males in the first mating. These results indicate that a high prevalence of SGH+ in *G. pallidipes* not only affects colony stability and performance but, in view of their reduced mating propensity and competitiveness, releasing SGH+ sterile male *G. pallidipes* will reduce the efficiency of a sterile male release programme.

Contributed Papers Viruses 4 Wednesday, 15:15 **132**

Molecular and Functional Analysis of ORF AMV133 Encoded by *Amsacta moorei* Entomopoxvirus (AmEPV)

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Amsacta moorei entomopoxvirus (AmEPV), belongs to Poxviridae, is an important insect virus. After analysis of complete genomic sequence of AmEPV, ORF AMV133 was suggested to be a putative triacylglyceride lipase gene. Transcriptomic analysis by RT-PCR indicated that, ORF AMV133 is transcribed at 6 hours post infection and has an early/late promoter. 5'-RACE analysis showed that transcription initiated at position –77, relative to the translational start site of this gene. To determine the limits of the putative promoters, upstream sequences of various lengths were cloned in front of a firefly luciferase reporter gene. The resulting plasmid constructs were tested in a dual lusiferase assay. The promoter activity was lost when the length of the sequence upstream of the translational start site was reduced from -82 to -21 nucleotides. Protein expression was performed in both bacterial and baculovirus expression systems. Expressed protein showed a significant level of lipase activity. Homolog recombination was used to generate an AMV133-knockout virus. However, the failure of producing recombinant virus shows that AMV133 is essential for virus productivity.

Contributed Papers Viruses 4 Wednesday, 15:30 **133**

The effect of expressing apoptosis-regulating genes on alphavirus replication in the mosquito vector *Aedes aegypti*

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Apoptosis is known to be a defense against some viruses in insects and mammals, but the role of apoptosis in mosquito immunity against arboviruses is just beginning to be explored. The mosquito *Aedes aegypti* is an important vector for yellow fever and dengue. Because of its ability to be engineered to express foreign genes, Sindbis virus (SINV; Togaviridae) was used to study the possible role of apoptosis in *A. aegypti* immunity against arboviruses. A series of infectious SINV clones based on the construct p5'dsMRE16ic was engineered to express pro-apoptotic or anti-apoptotic genes. A previous study had demonstrated that these SINV clones either induced or inhibited apoptosis as expected in cultured *Aedes albopictus* cells. Adult female *A. aegypti* were allowed to feed on blood containing the recombinant SINV clones or antisense controls, and virus infection was analyzed at various times post-infection in midguts by immunofluorescence (IFA) against the viral E2 protein. Viral replication was also monitored by titering the amount of infectious virus in individual mosquitoes. Virus clones expressing pro-apoptotic factors caused increased caspase activity and TUNEL staining in midgut compared to controls, indicating that apoptosis was stimulated by these virus clones. IFA and viral titer results indicated that infection with SINV clones expressing pro-apoptotic genes decreased the rate and spread of virus infection in the mosquito compared to controls. The results suggest that if apoptosis is induced in infected cells, it may be able to play a role in defense against arbovirus infection in mosquitoes.

Contributed Papers Viruses 4 Wednesday, 15:45 **134**

Replication biology of Providence virus (Family: *Carmotetraviridae*): a plant virus with an animal virus capsid that replicates in insects?

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Tetraviruses are small (+ve) ssRNA viruses that exclusively infect the larvae of Lepidopteron insects. Their capsids exhibit *T*=4 icosahedral symmetry and are composed of 240 copies of a single capsid protein, VCAP. The replicases of tetraviruses are remarkably diverse, forming three distinct groups representing three RNA virus replicase superfamilies: (1) the alpha-like replicase (*Helicoverpa armigera* stunt virus, HaSV), with characteristic methyltransferase, helicase and RNA-dependent RNA pollymerase (RdRp) domains; (2) the picorna-like replicase with a permuted RdRp domain (*Thosea asigna* virus, TaV) and (3) the carmo-like replicase of Providence virus (PrV), the only tetravirus that replicate in tissue culture. The PrV RdRp is closely related to plant viruses, (tombusviruses and umbraviruses) and contains a read-through stop signal that results in the translation of two overlapping replication proteins, p40 and p104. PrV also encodes a unique large ORF, p130, of unknown function. We have investigated the expression and subcellular localization of the PrV nonstructural proteins using fluorescence microscopy to localize PrV replication complexes, the subcellular distribution of which resembles that of the HaSV alpha-like replicase, which is targeted to detergent-resistant membranes derived from the endocytic pathway. As in the tombusviruses, the small (p40) replication protein interacts with the full length (p104) PrV replicase and this interaction is required for efficient subcellular localization. We also report on the expression and subcellular localization of p130, which undergoes 2A-like cleavage at its N-terminus to produce two translation products. We propose that p130 may have an analogous function to the movement proteins of umbraviruses.

Contributed Papers Viruses 4 Wednesday, 16:00 **135**

Suppression of RNA silencing by Wuhan Nodavirus

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RNA interference (RNAi) plays critical roles in cellular responses to viral infection in plants, insects and mammals. To escape from RNAi-mediated immunity, viruses encode viral suppressors of RNA silencing (VSRs) that sequester RNA duplexes and target protein components in the RNAi pathway. Although the endonuclease Dicers play critical roles in RNAi, the detailed mechanism by which VSRs antagonize Dicers remains unknown, and the interrelationship among diverse activities of VSRs evolved in RNA binding and interaction with protein components has not been investigated. Here, we show that from Wuhan Nodavirus (WhNV), B2 protein suppress *Drosophila* RNAi by the mechanism of interacting with Dicer-2 (Dcr-2) in addition to sequestering double-stranded RNA (dsRNA) and small interfering (siRNA). *In vivo* and *in vitro* studies further reveal that B2 binds to the RNase III and PAZ domains of Dcr-2 through its C-terminal region, thereby blocking the activities of Dcr-2 in cleaving dsRNA into siRNA and incorporating siRNA into RISC. Moreover, we uncovered an interrelationship among diverse activities of WhNV B2, showing that RNA binding to B2 could enhance the B2-Dcr-2 interaction by promoting B2 dimerization. All together, our findings establish a model on multiple suppression of *Drosophila* RNAi by targeting both RNA and Dcr-2 with WhNV B2, and lead to a speculation that the synergistic effect among diverse activities of WhNV B2 might be a general mechanism of multi-functional VSRs.

Contributed Papers Viruses 4 Wednesday, 16:15 **136**

The Drosophila RNAi machinery not only provides antiviral defense against RNA viruses but also DNA viruses.

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Insects and plants show an RNA interference-based antiviral immune response against RNA viruses. Viral dsRNA genomes or replication intermediates are processed into viral small interfering RNAs by a protein called Dicer-2 and this action compromises the infection. Whether the RNAi response also interferes with DNA virus infections remains an open question. In this contribution the role of RNAi in DNA virus infection was investigated using *Drosophila melanogaster* flies infected with *Chilo iridescent virus* (invertebrate iridovirus 6 or IIV-6) as a model. IIV-6 infected wild-type and RNAi mutant flies were analyzed for the presence of viral small interfering RNAs. It was found that these RNAs were not only produced in a Dicer-2 dependent manner, but were also derived from both strands of the viral genome in similar proportions. Furthermore the IIV-6 specific viral small interfering RNAs were derived from small defined regions of the viral genome. Mutated *D. melanogaster* flies unable to produce Dicer-2 and/or Argonaute-2 and therefore disabled in their RNAi response were more sensitive to IIV-6 infection. These data strongly suggest that RNAi also provides antiviral defense against DNA viruses in insects and that this defense is more universal to viruses than previously thought.

Contributed Papers Wednesday, 14:45 – 16:00

Fungi 3

Contributed Papers Fungi 3 Wednesday, 14:45 **137**

Elevated spring temperatures will impact fungal disease in gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), larvae

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The invasive forest pest, *Lymantria dispar,* reaches outbreak densities every 5-10 years. The fungal pathogen, *Entomophaga maimaiga*, infects *L. dispar* larvae throughout their range. Growth of these fungi, can be affected by temperature and humidity. Larval emergence in spring coincides with *E. maimaiga* resting spore germination suggesting that *L. dispar* movement into warmer regions and rising global temperatures may decrease the effectiveness of *E. maimaiga*. *L. dispar* larvae were exposed to continuous elevated temperatures of either 20, 24, 26, 28 or 30°C or were held at 24°C and exposed to 26, 28 or 30°C for either 24, 28 or 72 hours after inoculation. The effect of elevated temperatures and duration at elevated temperatures on larvae mortality and fungal sporulation were evaluated. Significantly fewer insects died and no insects sporulated when exposed to continuous 28 and 30°C. There was no effect of length of exposure on larval mortality at 26°C but larval mortality significantly decreased as the length of exposure to either 28 or 30°C increased. No insects that were exposed to ≥26, for 72h sporulated. These results suggest that rising ambient temperature of *L. dispar* habitat will reduce the efficacy of *E. maimaiga* to control *L. dispar* populations.

Contributed Papers Fungi 3 Wednesday, 15:00 **138**

Importance of spore discharge (numbers, distance and direction) of *Neozygites floridana* for epidemic development in *Tetranychus* populations

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This study aimed to understand factors that drive an epidemic development of *Neozygites floridana* in spider mite populations. Conidia discharged from *T. urticae* and *T. evansi* cadavers of Norwegian and Brazilian isolates were quantified in relation to distance and direction at different temperatures by placing fungus killed cadavers to sporulate either on coverslips or leaf disks. Temperature had a significant effect on sporulation and for the Norwegian isolate, more conidia were produced at 13°C (1886) and 18°C (1733) than at 23°C (1302). Most conidia from the Norwegian isolate were thrown at a distance of 0-0.6 mm from the cadavers and when placed facing downwards on the underside of a cover slip , half of the conidia were retained on the underside. When *T. evansi* cadavers killed by the Brazilian isolate were placed on the underside of a leaf, around 20% of the conidia were retained on the underside, most of them within a distance of less than 2.2 mm from the cadaver. The Brazilian isolate showed no differences in retention of capilliconidia between plants with high density of trichomes (tomato and eggplant) and low density (nightshade and pepper), and there was no difference in the number of capilliconidia produced by *N. floridana* among these host plants. A whole plant bioassay was conducted to reveal the plant location where *T. urticae* infected with the Norwegian isolate die and sporulate. Most of the cadavers were located at the lower to the middle part of the plant, while uninfected spider mites were more evenly distributed on the whole plant.

Contributed Papers Fungi 3 Wednesday, 15:15 **139**

Degeneration of wild-type and transgenic strains of *Beauveria bassiana*

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Strain degeneration denotes variation of production traits for microbial control, with main phenomena of decreased sporulation and virulence during subculturing, which causes great loss of spore production and field efficacy of *Beauveria bassiana*. A wild-type strain was subcultured on different media under different temperatures, humidity and light, with saltation frequency as an estimate of strain degeneration. The result showed that during subculturing, saltation happens with either the wild-type mother strain or its single spore isolates, with sporulation, growth rate and virulence on the Masson's pine caterpillar of all these isolates at the 5th generation varied significantly, suggesting that saltation happened by induction due to these culture conditions, but not spontaneously, and the variation mechanism involved heterokaryosis, heteroplasmosis, and mutation in either nuclei or mitochondria. Based on a complete mitochondrial genomic study on the mother strain and one of the sector isolate from one of its single spore isolates, variation distributed along the whole mtDNA sequence, but mainly in non-encoding region, with 75 base mutations, 203 base insertions and 173 base deletions detected and average variant rate up to 15.05%. Saltation also happened with a transgenic strain from one of the single spore isolate during subculturing. The transferred scorpion toxin gene *AaIT* was not lost, while sporulation and virulence declined faster than it wild-type grandmother strain and the single spore mother isolate. However, one of the sector isolate of the engineered strain mutated positively, with much slower decline of saltation rate, sporulation and virulence.

Contributed Papers Fungi 3 Wednesday, 15:30 **140**

Defense reactions of *Leptinotarsa decemlineata* larvae under combined treatments by fungus *Metarhizium anisopliae* s.l., bacteria *Bacillus thuringiensis tenebrionis* and organo-phosphorus insecticide

Olga. N. Yaroslavtseva, Ivan.M. Dubovskiy, Vadim.Yu. Kryukov, Elena.V. Surina, Galina.V. Benkovskaya and Viktor.V. Glupov

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The synergistic effect between *Metarhizium anisopliae* s.l. and *Bacillus thuringiensis* ssp. morisoni as well as between *M. anisopliae* and organophosphorus insecticide (OP) was observed in mortality of *Leptinotarsa decemlineata* larvae. We found the activation of nonspecific esterases and glutathione-S-transferase in haemolymph plasma and fat body of *Colorado potato* beetle compare to the control group in initial stage of mycosis (1-2 day after infection). However a decrease of the detoxificative enzymes activity of insects under treatment with *B. thuringiensis*, OP as well as joint treatments with M. anisopliae and *B. thuringiensis* or *M. anisopliae* and OP was registered. Moreover the significant decrease of encapsulation response was observed in larvae under infection with *B. thuringiensis* and joint treatments: *M. anisopliae + B. thuringiensis* or *M. anisopliae* + OP. We assume that the synergism under mixed treatments is the result of the suppression of defence systems by bacterial infection and insecticide treatment that can determine the susceptibility of insects to entomopathogenic fungi.

Contributed Papers Fungi 3 Wednesday, 15:45 **141**

Dietary effects on enzymatic immunity of migrating Mormon crickets to fungi and bacteria

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Migrating Mormon crickets lack proteins or carbohydrates in their diets. Protein deficiency reduces phenoloxidase (PO) based anti-fungal activity, whereas carbohydrate deficiency impedes anti-bacterial activity. To investigate the relationship between diet, movement, and immunity, we removed Mormon crickets from a migratory band and offered each one of five diet treatments: high protein, high carbohydrate, equal weight proteins and carbohydrates (P+C), vitamins only, or water only for one hour. We then attached a radio, returned them to the migratory band, and recaptured them 18-24 h later. Crickets fed protein moved the furthest, those without diet or only vitamins moved less, and those fed carbohydrates or P+C moved the least. Consistent with a previous study, anti-bacterial activity was greatest in those fed carbohydrates, and there was no difference between those fed water, protein, or P+C. Total PO activity also differed between treatments and was greatest in those fed protein and least in those fed water or vitamins only. To test for a hypothesized compromise between migratory and anti-bacterial activities, we removed crickets from the same migratory band and gave them one of four diet treatments: high P, high C, P+C, or vitamins only for 1 h. Hemolymph was drawn after 4 or 24 h. The effects of diet on total PO activity did not differ between captive and migrating crickets, but to have a dietary effect on anti-bacterial activity the crickets had to be migrating freely. Evidently migratory activity compromises anti-bacterial activity, whereas poor protein nutrition compromises PO activity independent of migratory behavior.

POSTER SESSION 2 Wednesday, 16:45 – 18:45

BACTERIA

Poster - Bacteria Wednesday 16:45 **B-18**

Characterization and colonization inside the plants *in vitro* of endophytic *B. thuringiensis* from sugar cane

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Bacillus thuringiensis is known worldwide due to its use in biological pest control. However, until now there are few reports about this endophytic microorganism. Being able to survive inside the plant is very special because this microorganism can act in the habitat where the conventional insecticides do not reach. Moreover, important ecological aspects, such as the ability of this organism to germinate, multiply and can spread in the environment have been poorly documented. This way, the aims of this study was to select and identify endophytic *B. thuringiensis* from inside of sugar cane, to evaluate the survival of these bacteria inside the corn and sugar cane plants *in vitro* and, finally, to characterize its Cry protein. Among all 800 endophytics bacteria from sugar cane just the isolated TC2.3.1R6 from the root was identified by 16S sequence as *B. thuringiensis.* By studying the survival of TC2.3.1R6 isolated inside the corn plant *in vitro*, we observed that the isolate did not colonize inside of corn roots or aerial parts. However, we observed that the multiplication of isolated inside of sugar cane roots. This isolated (TC2.3.1R6) synthesizes bipyramidals proteins and two secondary crystals, rectangular and round shaped. The amino acid sequence by mass spectrometry suggested the presence of Cry8Ba, Cry19Ba, Cry24Aa, Cry41Aa1 e Cry41Ab1 proteins. These results represent an advance to complete the gaps about the *B. thuringiensis* performances in the environment and also opens one more possibility for its use in biological control of insects that colonize inside of sugar cane roots.

Poster - Bacteria Wednesday 16:45 **B-19**

The effect of gamma sterilization on the insecticidal toxicity of engineered and conventional *Bacillus thuringiensis* strains

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Gamma irradiation generated by cobalt-60 (Co^{60}) effectively inactivates cells *via* ionizing radiation. Radiation sterilization is increasingly used for the sterilization of many pharmaceutical products and in food preservation by gamma rays.This study evaluates the effect of Co^{60} gamma radiation on the spore activity, toxicity and crystal structures of two engineered *Bacillus thuringiensis* (Bt) strains, TnX and TnY, and the reference Bt strain HD-1. We attempted to identify dosages of Co^{60} gamma radiation that would inactivate Bt spores but not affect its toxicity. In the radiation dosage range of 10 to 15 kilogray (kGy), there is no viable spore formation but no significant reduction of the efficiency of Bt against lepidopterous larvae. However, further SDS-PAGE results show that the components of the protoxin are affected by gamma radiation and that some bands are absent after treatment compared with the controls; the change in the protoxin band pattern depends on the type of Bt strain. Furthermore, the spore crystal structure of three Bt strains was studied with scanning (SEM) and transmission electron microscopy (TEM). The results show that there are no changes in the size or shape of the treated Bt spores and crystals compared with the controls, and the use of gamma radiation is effective to inactivate the spores of engineered Bt strains while preserving stable Bt toxicity against the target insect larvae. The sterilization of the engineered strain may be essential for acceptance by the general public.

Poster - Bacteria Wednesday 16:45 **B-20**

The importance of antibiosis for the successful reproduction of *Bacillus thuringiensis* in insects

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While the relationship between intestinal bacteria and the virulence of *B. thuringiensis* has been controversial, the balance of evidence suggests that *Bt* does not generally require additional microbes for full expression of virulence. Given that *Bt* and midgut bacteria can both invade the haemocoel, a more plausible ecological scenario is that these bacteria undergo intense competition for the resources contained in the host. I tested this hypothesis by investigating: (1) the distribution of genes involved in the production of the antibiotic zwittermicin A in the *Bacillus cereus* group and (2) using zwittermicin A knock out mutants to investigate the role of this antibiotic in reproduction of *Bt* in insects (*Plutella xylostella*) cultured with and without instestinal bacteria.

Poster - Bacteria Wednesday 16:45 **B-21**

Effects of gut bacteria to the insecticidal activity of *Bacillus thuringiensis* on *Helicoverpa armigera*

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Bacillus thuringiensis is widely used in pest control because of the highly expressed special toxic insecticidal crystal proteins generated during its growth. Recent research on insecticidal mechanism of *B. thuringensis* shows that some symbiotic bacteria are necessary for insecticidal activity of *B. thuringiensis*, but there are some researchers argue about the gut bacteria model. In this study, we chose *Helicoverpa armigera* as the experimental insect, tested whether symbiotic gut bacteria of *H. armigera* play a significant role in insecticidal activity of Cry1Ac10 crystal protein of *B. thuringensis* toward the host. Our test showed that purified *B. thuringensis* toxin protein was fully pathogenic to larvae that were continuously exposed to antibioties. Aseptic larvae that were continuously exposed to antibiotics prior to bioassay died more quickly than larvae reared normally. Our result showed that gut bacteria are not necessary for insecticidal activity of *B. thuringensis*, instead these bacteria protect *H. armigera*. And *H. armigera* midgut bacteria 5 affected the sensitivity of insecticidal crystal protein toward *H.armigera* most by approximately 20%.

This work received the financial support from the National Natural Science Foundation of China (Grant No. 30871672)

Poster - Bacteria Wednesday 16:45 **B-22**

Immune response of *Galleria mellonella* (Lepidoptera, Pyralidae) larvae during bacterial infection by *Bacillus thuringiensis*

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The mechanisms of insects resistance to the bacterium *Bacillus thuringiensis* (Bt) based on the activity of immune response during infection of Bt were studied. The cellular and humoral immune reactions of *Galleria mellonella* have been investigated during sublethal and half-lethal natural gut bacterial infection

by *Bt,* in order to examine the role of the immune response in protecting insects during intestinal infections. The elevated activity of the phenoloxidase and lysozyme-like activity of the haemolymph, and increased level of the phagocytic activity of haemocytes and encapsulation response of infected insects have been shown under the sublethal (LC15) bacterial infection Bt on the second and third days after the treatment. At the half-lethal concentration of Bt (LC50) we have detected the significant elevated activity of the phenoloxidase and lysozyme-like activity of the haemolymph, but the decreased coagulation index and activity of the phenoloxidase in haemocytes of infected insects. The cellular and humoral immune reactions as a part of induced resistance of insect under intestinal bacterial infection Bt will be discussed.

Poster - Bacteria Wednesday 16:45 **B-23**

Characterization of *vip* genes and toxicity of *Bacillus thuringiensis* against *Spodoptera frugiperda*

Camila da Silva Fernandes[2], Thais Barros Rodrigues[1], Rosane Bezerra da Silva[1], Arthur Augusto Gonçalves Torres[2], André Henrique Campelo Mourão[2], Kátia Gisele Brasil Boregas[3] and Fernando Hercos Valicente[3]

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The objective of this study was to characterize 62 strains of *B Bacillus thuringiensis* for the presence of genes *vip* and its toxicity towards *Spodoptera frugiperda*. These genes were amplified with *vip2* and *vip3-type* primers. For mortality bioassays, a negative control (distilled water) and a positive control (344 *Bt* sv *tolworthi*) were used. Plastic cups (50 mL) with artificial diet were inoculated with 150 µL of suspension of spores and crystals. After the excess of the Bt suspension evaporated, larvae of the second instar were individualized, with a total of 48 replicates per treatment (strain), and checked after one week. It was observed that only *vip3-type* gene, was found in the strains examined, with a low frequency of 9.67%, and *vip2* gene was not found in these strains, 56% of the strains amplified products for *vip3Aa* gene , 18% for *vip3D* gene, and 1.61% for *vip3Aa´* gene. Most of the strains examined was not significantly different from the lower mortality (0%). A few strains showed mortality rates between 6-28%. These results suggest that *vip3* is the most common among those evaluated. However, they are a powerful tool in Lepidoptera insect control.

Poster - Bacteria Wednesday 16:45 **B-24**

Identification of Coleoptera and Lepidoptera-specific *vip* genes in Argentinean and exotic *Bacillus thuringiensis* strains

Diego Sauka, María Inés Onco, Sonia Rodríguez, Melisa Pérez and Graciela Benintende

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The Vegetative insecticidal proteins (Vip) are produced during the vegetative growth stage of *Bacillus thuringiensis*. These virulence factors emphasize the potential benefit of its use in resistance management strategies, since the discovery of new *vip* genes could be useful as tools against resistant pests. The aim of this work was to identify *vip1*, *vip2* and *vip3* genes in 86 *B. thuringiensis* strains obtained from the IMYZA-INTA Bacterial Collection. Pairs of primers derived from conserved regions and from sequence alignment consensus were used to detect these genes by PCR. Subsequently, positive strains were characterized by RFLP using specific restriction enzymes in order to identify known and novel subclasses of these genes. Seventy eight percent (39/50) of the Argentinean and 33.3% (12/36) of the exotic strains were positive for the tested genes, with higher frequency for strains harboring *vip3* genes alone (38 native and 6 exotic strains respectively). By PCR-RFLP, 10 polymorphic profiles were observed, indicating the presence of different alleles, and, therefore, of different subclasses of *vip* genes.

Poster - Bacteria Wednesday 16:45 **B-25**

Asparagine substitution in block 3 of *Bacillus thuringiensis* crystal protein Cry5Ba improved the crystal solubility and increased the toxicity against *Caenorhabditis elegans*

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The crystal proteins from *Bacillus thuringiensis* were widely used for their specific toxicity against insects and nematodes. The highly conserved sequence blocks take an important role in Cry protein stability and flexibility, the basis of toxicity. The block 3 in Cry5Ba subfamily has a shorter sequence, only 12 residues, and more asparagine residues than that of others which harbor about 48 residues but only one asparagine. Based on the theoretical structure model of Cry5Ba, all three asparagines in block 3 are closely located in the interface of putative three domains, implying their probable importance in structure and function. In this study, all three asparagines in Cry5Ba2 block 3 were individually substituted with alanine by site-directed mutagenesis. The wild-type and mutant proteins were over expressed and crystallized in acrystalliferous *B. thuringiensis* strain BMB171. However, the crystals formed in one of the mutants, designed as N586A, abnormally disappeared and dissolved into the culture supernatant once the sporulation cells lyzed, while Cry5Ba crystal and the other mutant crystals were stable. The mutation N586A crystal, isolated from sporulation cells by ultrasonic process, was found to be easily dissolved at wide range of pH value (5.0-10.0). Moreover, the nematode toxicity assays showed that the mutant N586A demonstrated an elevated activity nearly 9 times and damaged the nematode intestine more efficiently than the native Cry5Ba2. These data support the presumption that the amide residue Asn586 in the interface of domains might adversely affect the protein flexibility, solubility and resultant toxicity of Cry5Ba.

Poster - Bacteria Wednesday 16:45 **B-26**

Characterization of an active partition system for the *Bacillus sphaericus* mosquitocidal plasmid pBsph

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Plasmid pBsph encodes binary toxins (BinA/BinB), which are toxic toward mosquito larvae. Although the aspects of Bin toxins have been studied extensively, very little is known about the replication and partition of this plasmid in *Bacillus sphaericus*. In this study, a 2.4 kb DNA fragment as the minimal replicon (minireplicon) of pBsph was identified, consisting of ORF189 and ORF188 in an operon. Mutational analysis showed that the two ORFs are indispensable for the replication of pBsph in *B. sphaericus*. The knocking-out of native ORF188 could result in plasmid-curing of the pBsph from *B. shparicus* C3-41. It was observed that the minireplicon could replicate in *B. thuringiensis*, *B. cereus* and *B. sphaericus* with a low copy numbers (2 to 3 copies per chromosome). Further analysis indicated that ORF189 contains a winged-helix DNA binding domain in its C-terminal region and presents as a homodimer in solution, ORF188 contains a tubulin signature motif (GGGTGTG), and a mutation (T114A) in this motif abolished its replication activity. Electrophoresis mobility shift assays (EMSAs) demonstrated that His_6-ORF189 binds specifically to the region upstream of minireplicon, while His_6-ORF188 binds to the ORF189-DNA complex. In addition, it was shown that His_6-ORF188 is a GTPase, and the mutated protein ORF188-T114A could severely impair its GTP hydrolysis rate (about 8 fold lower). Furthermore, it was observed by electron microscopy that ORF188 can assemble into long filaments in a GTP-

dependent manner, while mutated protein T114A impaired the depolymerization of the filaments. Taken together, our results demonstrated that the ORF189-binding region, DNA-binding ORF189 and GTPase ORF188 composes of an active partition system for the replication and accurate segregation of pBsph in *B. sphaericus*.

Poster - Bacteria Wednesday 16:45 **B-27**

Generation of mariner-based transposon insertion mutant library of *Bacillus sphaericus* 2297 and investigation of genes involved in sporulation and mosquito-larvicidal crystal protein synthesis

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Bacillus sphaericus has been used with great success in mosquito control programs worldwide. Under conditions of nutrient limitation, it undergoes sporulation via a series of well defined morphological stages. However, only a small number of genes involved in sporulation have been identified. To identify genes associated with sporulation, and to understand the relationship between sporulation and crystal protein synthesis, a random mariner-based transposon insertion mutant library of *B. sphaericus* strain 2297 was constructed and seven sporulation-defective mutants were selected. Sequencing of the DNA flanking of the transposon insertion identified several genes involved in sporulation. The morphologies of mutants were determined by electron microscopy and synthesis of crystal proteins was analyzed by SDS-PAGE and Western blot. Four mutants blocked at early stages of sporulation failed to produce crystal proteins and had lower larvicidal activity. However, the other three mutants were blocked at later stages and were able to form crystal proteins, and the larvicidal activity was similar to wild type. These results indicated that crystal protein synthesis in *B. sphaericus* is dependent on sporulation initiation.

Poster - Bacteria Wednesday 16:45 **B-28**

Characterization of a *Bacillus thuringiensis* strain native from Argentina toxic against mosquito species

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Mosquitoes (Culicidae) are blood-sucking insects of public health importance, mainly because of their importance as vectors of many hazardous diseases, like dengue, yellow fever, malaria, several types of encephalitis including West Nile fever, and lymphatic filariasis. The application of different strategies to management of mosquito populations is essential for the control of these viral diseases. In a previous work, we characterized a *Bacillus thuringiensis* isolate native to Argentina (FCC 41) that exhibits mosquitocidal activity and harbourgs he protein Cry24Ca in its parasporal body. Here we describe the presence of an Orf2-like sequence downstream the secuence *cry24Ca*. On the other hand, we detected a new protein, Cry 50-like by biochemicals techniques like sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE) and MALDI-TOF mass spectrometry. We used a PCR-based strategy for amplification of DNA fragments with degenerated oligonucleotides homologous to *cry50-like* genes, followed by Tail-PCR methodology, in order to clone and sequence the entire gene. BLASTp analysis indicated that the new sequence is related to Cry 52 protein type. Moreover, in order to extend the toxicological spectra of FCC 41 strain, toxic against *Aedes aegypti* mosquito, we performed bioassays against other three mosquito species: *Culex pipiens*, *Cx. apicinus* and *Ochlerotatus albisfaciatus.* A high larvicidal activity was observed in all cases. Thus, our findings expanded the toxicological spectra of this native strain and suggest that it could be used as a microbial insecticide for the control of several vector mosquitoes species.

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Poster - Bacteria Wednesday 16:45 **B-29**

Enterotoxigenic and psychrotrophic but not entomo-pathogenic properties of environmental *Bacillus thuringiensis* isolates correlate with the phylogenic relatedness.

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In the present study we used pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) to assess the genetic structure and phylogeny of environmental isolates of *Bacillus thuringiensis*, and to determine whether *B. thuringiensis* isolates possessing features common with the other members of the *B. cereus* complex create separate phylogenetic clusters. To address these questions we analysed (i) the ability of the isolates to grow at low temperature, a cardinal feature of psychrotrophy as exemplified by strains of *Bacillus weihenstephanensis;* (ii) the presence of enterotoxin genes homologues of *B. cereus* responsible for foodborne diarrheal syndrome; and (iii) the distribution of the pXO1- and pXO2-like replicons of *Bacillus anthracis*. A total of 47 *B. thuringiensis* isolates from soil, 24 from Central Lithuania and 23 from North-Eastern Poland, were screened. *B. thuringiensis* HD-1, HD-133, and HD-567, *B. weihenstephanensis* DSMZ 11821, and *B. cereus* ATCC 14579 were used as reference strains. The sequences of the seven housekeeping genes of these isolates were compared to allelic sequences available at the MLST *B. cereus* database. Sequence variability for each fragment varied from 9.1% (*glp*) to 21.4% (*ilv*) with the number of alleles per locus ranging from 9 to 18, respectively. For each locus, except the *gmk*, the number of alleles among Polish isolates was slightly higher when compared to isolate recovered fom Lithuania. Among the 47 isolates, a total of 42 STs (sequence type) were distinguished. The psychrotrophic isolates clustered in one branch in MLST analysis and PFGE fingerprint. Similarly, the *cyt* gene clustered together, while the genes connected with the *B. anthracis* plasmid replicons and those encoding *Cry* toxins were present in different branch of the MLST dendrogram indicating horizontal gene transfer between the *B. cereus* group members.

Poster - Bacteria Wednesday 16:45 **B-30**

Experimental evidence supporting the pore-forming model of the mechanism of action of 3d-Cry toxins

Isabel Gómez, Carlos Muñoz-Garay, Liliana Pardo, Helena Porta, Claudia Rodriguez, Jorge Sanchez, Luis E.Zavala, Violeta Matus, Leivi Portugal, Josue Ocelotl, Fernando Zuñiga, Daniela Carmona, Mario Soberón and Alejandra Bravo.

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Bacillus thuringiensis bacteria are insect pathogens that produce different Cry and Cyt toxins to kill their hosts. Here we will present evidence that supports the pore forming model of the mechanism of action of the three-domain Cry (3d-Cry) toxins. This model involves sequential interactions with several insect midgut proteins, facilitating the formation of a pre-pore oligomeric structure and inducing its insertion into the membrane, forming a pore that kill midgut-cells. We present her strong evidence supporting this model :

1. Interaction with cadherin APN and ALP receptors depends on the oligomeric state of the toxin. Mutant toxins affected in the interaction of either the monomeric or oligomeric Cry1A toxins with receptors are non-toxic.

2. Pull down experiments performed with monomeric or oligomeric structures of the toxin showed that monomeric toxin have a more stable interaction with cadherin while oligomeric structure has a more stable interaction with APN.
3. Analysis of apparent dissociation constants showed that monomeric toxin has a high affinity interaction with cadherin while oligomeric structure has a high affinity interaction with APN and ALP receptors
4. Mutant toxins in helix alpha-3 are affected in oligomerization and are non-toxic.
5. Mutant toxins affected in helix alpha-4 are affected in pore formation and membrane insertion. These mutants are still able to oligomerize and make hetero-oligomers with wild type toxin showing a dominant negative phenotype since they inhibit toxicity of Cry1A as anti-toxins.
6. Pore activity of oligomeric structures is more efficient than monomeric toxin, inducing stable pores with high open probability. In contrast monomeric toxin produces pores that show much lower open probability.
7. Cry1AMod toxins deleted of helix alpha-1 are able to form toxin oligomers in absence of receptors, and are active against different resistant populations affected in cadherin but also affected in other proteins such as APN or ABC transporter.
All these data support the model of pore formation and the hypothesis that toxin oligomerization is a limiting-step in Cry toxins insecticidal activity.

Poster - Bacteria Wednesday 16:45 **B-31**

***Bacillus thuringiensis* and plants: an *in vitro* model to study interactions**

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Agrochemical inputs have contributed to an unprecedented increase of productivity in the last decades, however, their environmental impact highly compromises sustainability. Therefore, alternative methods are urgently needed. Beneficial plant-associated bacteria play a key role in plant health and growth both in natural and managed ecosystems, and the majority of them derive from the soil environment. Here we present a method to investigate interactions of the model plant *Arabidopsis thaliana* and rhizobacteria, with special interest in the biocontrol agent *Bacillus thuringiensis* (Bt). Bt is a well know entomopathogen, it is a soil-borne bacterium although its ecology is not well understood, as it has been isolated from many other habitats. There is increasing evidence that Bt interacts with plants under and above ground, epi- or endophytically and may even exert a plant growth promoting effect. We have developed an *in vitro* microcosm, reproducibly to screen and analyse the root colonisation competence of a collection of environmental Bt isolates, together with Bt reference strains and other well reported plant growth promoting rhizobacteria. Identification of suitable strains and a deeper understanding of their interactions with plants will increase our knowledge of Bt's ecology and potentially will open new perspectives on the agricultural use and delivery of this important biocontrol agent.

Poster - Bacteria Wednesday 16:45 **B-32**

***Bacillus thuringiensis* strains for pest control in Brazil**

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The aim of this study was to select *Bacillus thuringiensis* strains for control of important pests in Brazil. Selective and dose bioassays were performed. For selective ones 56 strains was using for *Anticarsia gemmatalis*, 50 for *Pseudoplusia includens*, 54 for *Spodoptera frugiperda*, 49 for *Spodoptera eridania*, 15 for *Spodoptera cosmioides*, 34 for *Plutella xylostella*, 56 for *Chlosyne laciniasa undersii*, 45 for *Anthonomus grandis* and 40 strains for *Alphitobius diaperinus*. In the selective bioassays the strain BR37 caused mortality of 98% in *P. includens*, 93% in *C. lacinia saundersii*, 96% in *S. frugiperda*, 95,5% in *S. eridania* and 80% in *S. cosmioides*. Strain S1269 cause high mortality in *C. lacinia saundersii* (85%), *S. frugiperda* (90%), *A. gemmatalis* (89%) and *P. xylostella* (100%). Strain S1265 cause also high mortality that was 100% in *P. includens*, 96% in *S. frugiperda*, 90% in *P. xylostella* and 82% in *A. gemmatalis*. S1302 strain caused mortality of *P. includens* (100%), *S. eridania* (90%) and *A. gemmatalis* (80%). For *A. grandis the strains* S 1989, S 1122, S 1342, caused respectively 100%, 88% and 85% mortality. The best strain for *A. diaperinus* was BR34 however with low mortality, only12%. In the dose bioassays strains BR12 and BR83 caused the lowest LC_{50} for *P. includens*. For *C. lacinia saundersii* the most virulent strains were S1302, S1269, S1450, and BR87. For *S. frugiperda* and *S. eridania* strain BR 58 was the most virulent. For *A. grandis* S1122, S1989 and S1269 were the most virulent. (Grants: CNPq and CAPES, Brazil)

Poster - Bacteria Wednesday 16:45 **B-33**

Shell disease by *Vibrio* sp. in grapsid crabs from Bahía Blanca estuary, Argentina

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In a survey carried out to increase the knowledge about pathogens of crustaceans in Argentinean wetlands, several species of shrimps and crabs were studied. In 5% of the grapsid crabs *Neohelice granulata* (n = 36) and *Cyrtograpsus angulatus* (n = 65) collected in Bahía Blanca estuary, erosive lesions of until 5 mm were observed in ventral and dorsal surfaces of shells. Some crabs with lesions were fixed in 10% formalin for histological studies. Others lesions were scraped in aseptic conditions and the material was spread in plates with TCBS and incubated at room temperature for 24-48 hs. Positive plates showed round, yellow, and bright colonies integrated by Gram-negative bacteria. DNA extracted from this colonies was amplified by PCR using general primers for 16s rDNA segment and then sequenced. In histological sections of the cuticle very extensive foci were observed. Lesions were characterized for extended erosions throughout the epi-, exo-, and endocuticle, wich was often melanized. In the foci, bacteria, protozoans, and debris were observed. DNA sequences were compared to the ones deposited in GenBank (BLASTN) and the Ribosomal Database Project (RDP). We observed a close relationship with several *Vibrio* spp. Some of these species had been reported in association with crustacean culture: *V. alginolyticus* (96% BLASTN), *V. furnissii* (95% BLASTN), *V. vulnificus* (96% BLASTN), *V. fluvialis* (95% BLASTN), *V. paccinii* (85% RDP), and *V. parahaemolyticus* (86% RDP). Biochemical reactions will be necessary for the final identification of the causative agent of this shell disease.

Poster - Bacteria Wednesday 16:45 **B-34 STU**

Cloning and expression of a novel *cry1I* gene from *Bacillus thuringiensis* isolates and its toxicity against *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae) and *Helicoverpa armigera* Hübner (Noctuidae: Lepidoptera)

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Biocontrol of pests via *Bacillus thuringiensis* (Bt) δ-endotoxins represents the most successful use of a biological control agent to date. The most notable characteristic of *Bacillus thuringiensis* is its ability to produce insecticidal proteins. More than 300 different proteins have been described with specific activity against insect species. The six isolates of *Bacillus thuringiensis* from Andaman and Nicobar Islands which were previously characterized by PCR analysis for the presence of Coleopteran active *cry* genes were used for *Cry1I* full length gene amplification. A 2.16-kb DNA fragment of *Cry1I* gene was PCR amplified, cloned in expression vector pQE 80 L, and then used for transformation of *E. coli M15* cells. The optimum expression was obtained with 1 mM IPTG at 37^{0}C for 3 h. The sequence of the cloned crystal protein gene showed almost complete homology with a *Cry1I* toxin gene from *Bacillus thuringiensis* var. *kurstaki*, with scattered mutations in the toxic region. The deduced sequence of the protein has homologies of 91.0% with *Cry1I* and *Cry1Ia*, and 98.0% with *Cry1Ib*.Cloning of this gene may help to overcome the increasing resistance of pests to currently used insecticides. Based on the results obtained, the PCR method may be a valuable and reliable tool for specific detection and identification of *cry1I* genes. The toxicity of Bt recombinant protein was determined against first instar larvae of *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae) and Adults; *Helicoverpa armigera* Hübner (Noctuidae: Lepidoptera) at 310µg/mL and 15.5µg/mL respectively. The novel *cry*1I gene will be an important resource in constructing genetically engineered bacteria and transgenic plants for biocontrol of insect pests and Bt based biopesticidal formulations, aiming to reduce the use of chemical insecticides.

POSTER SESSION 2 Wednesday, 16:45 – 18:45

FUNGI

Poster - Fungi Wednesday 16:45 **F-20**

Sclerotinia sclerotiorum white mold inhibition by volatile metabolites of entomopathogenic fungi

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The effect of volatile metabolites produce by mycelia, of entomopathogenic fungus *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium* sp., *Nomuraea rileyi and Paecilomyces lilacinus* also used for root nematodes biological control, on growth inhibition of pathogen *Sclerotinia sclerotiorum* the white mold, was evaluated. Experimental design was completely randomized with six treatments and five replications. The tests were developed using the methodology that positioning the Petri dishes bottom one over the other, both containing PDA culture medium. The entomopathogenic fungus were inoculated with 0,1 mL of spores/conidia (10 mL of sterile distilled water + one colony of entomopathogen disc) on plates of 9 cm diameter containing PDA. After incubation and complete mycelia growth of the entomopathogen colony on the culture medium, the Petri dish cover was removed and positioned on top of another Petri dish bottom with PDA medium, which was placed a colony disk of *S. sclerotiorum*, in the center. Thus, the set comprised of two Petri dish bottom one over the other (bottom: entomopathogens and cover: *S. sclerotiorum*), were sealed with plastic film. Control plates were made only with *S. sclerorotiorum* discs. Evaluation was performed after 72 hours incubation, when colonies diameter were measure and comparing the entomopathogens treatments plates and control plates. *P. lilacinus* and *S. sclerotiorum* was the only treatment that show differences from the control and from the others treatments promoting 76% ± 10 mycelial growth inhibition of *S. sclerotiorum* proving the effect of possible volatile metabolites produced by the entomopathogen.

Poster - Fungi Wednesday 16:45 **F-21**

Influence of successive *in vitro* cultivation of *Beauveria bassiana* (Bals.) Vuill on virulence to *Alphitobius diaperinus*

Patricia H. Santoro, Pedro M. O. J. Neves, Janaina Zorzetti and Kelly C. K. Silva

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The successive subculture *in vitro* can affect the entomopathogenic fungi quality. The aim of this study was to assess the effect of successive subculture of *Beauveria bassiana in vitro* under different nutritional conditions on virulence to the *Alphitobius diaperinus*. PDA (potato dextrose agar) and MAD (medium consisting of *Alphitobius diaperinus* adult insects) medium were used. The fungus (Unioeste 4) was initially inoculated into the host (1st(A)), subcultured 17 times in different media, inoculated into the insect for second time and subcultivated again in different media (1st(B)). For this test conidia of the 1st(A), 4th, 8th, 12th, 17th and 1st(B) (8 × 10^6 conidia ml^{-1}) subcultures were selected and sprayed on *A. diaperinus* adults insects. The assessment was realized on the 10th day when the dead insects were placed in climatized chamber (25 ± 1°C) for five days to confirm the mortality by the pathogen. The successive subculturing and the nutritional conditions of the medium affected the fungus virulence in the insect. The subcultures in PDA caused reduction in virulence, however, it could be restored after the second inoculation in the host. The MAD medium besides providing more virulent conidia, also favored the virulence maintenance after 17 subcultures. That maintenance can be associated with the nutritional medium aspects. Thus, it is necessary to identify the subculture conditions that preserve the virulence without increasing production costs. In this sense, the fungus inoculation into the host as well as the use of different culture media can be considered as an alternative.

Poster - Fungi Wednesday 16:45 **F-22**

Entomophthorales fungi (Zygomycetes) pathogens of aphids (Hemiptera: Aphididae) associated with cereal crops in Argentina

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The aphids represent for cereal production in Argentina on of the main causes of economic loss. Control of aphids has been done predominantly by using chemical insecticides. However this practice has caused problems for the environment, human health, and some species of aphids have developed resistance to insecticides. As natural enemies of aphids, Entomophthoralean fungi have been found to be important mortality factors in the field. The overall objective of this study was to do a survey and identification of entomophthoralean fungi species pathogenic to aphids of cereal crops. The studies were conducted in crops of *Triticum aestivum* L. (wheat), *Avena sativa* L. (oats) y *Sorghum bicolor* L. Moench (sorghum) during two consecutive years with weekly frequence in West region of Santa Fe, Argentina. Six aphid species were recorded as hosts of Entomophthorales fungi: *Rhopalosiphum maidis* (Fitch),

Rhopalosiphum padi (Linnaeus), *Rhopalosiphum rufiabdominalis* (Sasaki), *Shizaphis graminum* (Rondani), *Sitobion avenae* (Fabricius) y *Sipha maydis* Passerini. Three species of Entomophthoralean fungi were found infecting these species of aphids. *Pandora neoaphidis* (Remaudière & Hennebert) Humber and *Zoophthora radicans* (Brefeld) Batko (Entomophthorales: Entomophthoraceae) were the dominant pathogens of aphids during 2010 y 2011 respectively. However *Neozygytes fresenii* (Nowakowski) Remaudière & Keller (Entomophthorales: Neozygitaceae) was only recorded during the second year of the survey. Entomophthoralean fungal infections occurred mostly in autumn-winter season, coinciding with periods of high relative humidity and relatively low temperatures. This study is the first report of Entomophthoralean fungi infecting aphid pest in cereal crops in Argentina.

Poster - Fungi Wednesday 16:45 **F-23**

Morphological characterization of *Hirsutella citriformis* species infecting *Diaphorina citri* Kuwayama in Mexico

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The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is a vector of citrus greening disease, which is the most serious disease of citrus. Citrus greening disease caused by the bacterium *Candidatus* Liberibacter asiaticus renders fruit unusable and kills the trees. Recently were found insects identified as *Diaphorina citri*, infected with fungus in various states of Mexico. These species of fungus were isolated, cultured on potato dextrose agar and after monosporic cultures were obtained of these strains. In this work we described the microscopic characterization of these native strains from Tabasco, Colima, San Luis Potosí and Campeche states. The microcultures obtained were incubated at 26 ± 1 during 20 days. The strains isolated through different techniques presented slow growth, with mycelim composed by delicate hyphae measuring 11.6 to 16.4 µm long and 1.18-1.88 µm diameter, phialides with spherical or oval base, of 30.7-40.9 µm long, with typical single elongated conidia (cymbiform) or fusiform, of 5.8-5.9 X 1.4-1.9 µm size, which were covered by an ovoid or lemon-shaped, mucilaginous, water–soluble layer measuring 7.8-8.1 X 5- 5.9 µm. The morphological characterization indicated that the new isolates were related to *Hirsutella citriformis* Speare, which represents a potential alternative as a control agent for the Asian citrus psyllid in Mexico.

Poster - Fungi Wednesday 16:45 **F-24**

***Metarhizium anisopliae* and *Beauveria bassiana* blastospores obtained in submerged culture against *Aedes aegypti* larvae and adults.**

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Three strains of *M. anisoplae* and three strains of *B. bassiana* were propagated in three liquid media containing casaminoacids, soybean flour or sunflower seed flour with shaking for three days. The *Metarhizium anisopliae* strains cultivated in the three liquid media showed yields of blastospores between 1.9 and 4.03 x10^7 blastospores/ml, where the comparison of mean numbers of blastospores produced by *M. anisopliae* indicated that strain IB-Ma-2 produced more blastospores than did the other strains of *M. anisopliae*. The *B. bassiana* strains showed greater numbers of blastospores compared to the strains of *M. anisopliae*, in the same culture media, with values of 0.2-8.2 x 10^8 blastospores/ml. The liquid cultures of *M. anisopliae* tested against late third stage larvae of *Aedes aegypti* showed differences in mortality. The comparison of mean mortality rates indicated that strain IB-Ma-2 of *M. anisopliae* showed significantly greater mortality than strains IB-Ma-4 and IB-Ma-1. The blastospores of *Beauveria bassiana* propagated in the three culture media showed little mortality against *Aedes aegypti* larvae, with values of 1-15%. The strain IB-Ma-2 of *M. anisopliae* propagated in casaminoacids medium showed an LC_{50} of 9.58 x 10^5 blastospores/ml against mosquito larvae at 5 days postapplication. In bioassays against *A. aegypti* adults, blastospores of *M. anisopliae* IB-Ma-2 caused 27% mortality at 10 days postapplication.

Poster - Fungi Wednesday 16:45 **F-25**

Relative Production of Metarhizium Propagules and their Potential as Human Pathogens

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The two phase (liquid, solid) fermentation method is routinely used to mass produce Metarhizium conidia for use as the active ingredient for biological control of insects. An accepted understanding is that the use of blastospores for inoculating solid media is preferable because their rate of growth is logarithmic. For isolates that produce few, if any blastospores, we wondered if liquid culture-generated mycelia (LCGM) had a similar rate of growth to blastospores. We found that the radial growth rate of colonies grown on potato dextrose agar point-inoculated with each of blastospores, LCGM, and condia were identical. However, under aerobic fermentation on barley, harvested conidia yields were twice as high on substrate inoculated with liquid suspensions of blastospores, compared to substrate inoculated with liquid suspensions of conidia. Furthermore, conidia yields of different isolates grown on PDA were not reflected in a relative sense to yields produced by barley. We also found that for two isolates, F52 (internationally commercialized) and MetaFHIA (from Honduras), LCGM always grew and occasionally sporulated at 37C on PDA, rendering it as being potentially pathogenic to humans.

Poster - Fungi Wednesday 16:45 **F-26**

Distribution of Metarhizium species in relation to ecoregions of the North American subcontinent

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A systematic examination on occurrence and distribution of Metarhizium on a large geographic scale was conducted. We sampled soil from both agricultural and natural sites ranging from north central-British Columbia and Alberta in the north to central Oregon in the south, and along the Pacific northwest coast in the west to the Canadian prairies in the east, and acquired Metarhizium isolates using Galleria bait technique. The species of each isolate was identified by sequencing the ITS and the entire 5' tef1 region, and their spatial occurrence mapped overlaying Level III Ecoregions of North America. A total of 82 natural sites and 61 agricultural sites were sampled, paired

within close proximity in most cases. Metarhizium was found at 20/82 (24%) natural sites, and 26/61 (43%) of agricultural sites. Four unique Metarhizium species were identified, with M. brunneum (at 125 sites) being the most common, followed by M. robertsii (23 sites), and M. flavoviride v. pemphigi and M. guizhouense found at one site each. In North America, precautionary policies regarding the release of entomopathogens for testing as biocontrol agents require special permissions for isolates foreign to new ecological regions ('ecoregions'). We believe that our future work to examine both the species distribution and AFLP-based genetic variability within species in relation to ecoregions will provide scientific information so that these policies might be reconsidered with a better understanding of entomopathogen ecology.

Poster - Fungi Wednesday 16:45 **F-27**

Response of *Beauveria bassiana* and *Metarhizium* spp. vegetative cultures to transient high temperatures.

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Thermal tolerance is an important characteristic in the selection of entomopathogenic Hypocreales for a specific target pest, with the important temperature range being determined by the host's environment. Almost all studies of fungal responses to temperature have used constant temperatures to characterize isolates. But because of "behavioral fever," grasshoppers and Mormon crickets present a different scenario, one of daily transient high body temperatures of 35-41° C. Typically, on the North American Plains, a fungus infection in a grasshopper faces ~6 hours of elevated temperatures. My study examined the in vitro responses of 197 isolates of *Beauveria bassiana* (isolated from grasshoppers), 108 *Metarhizium* spp. (isolated from soil), plus the 2 commercial *M. acridum,* to 37° or 41° C. for 6 hr, after which the cultures were returned to an optimal 27° C. Past research has assumed that these fungi resume normal growth when temperatures fall to below their upper threshold. However all the isolates tested, except the *M. acridum*, demonstrated a delay in resumption of vegetative growth after a *single* exposure to 41° C. This delay lasted from 24 to 166 hr depending on the isolate. After exposure to 37° C., 62 *Beauveria* and 96 *Metarhizium* had no delay, but the remaining 149 isolates had a delay of 3 to 59 hr. Once normal radial growth had resumed, the growth rate varied considerably in comparison to the growth at the optimal temperature of the isolate. Some isolates grew significantly faster, others slower, and still others at the same, "normal" rate.

Poster - Fungi Wednesday 16:45 **F-28**

HURRICANE WARNING! How changed nomenclatural rules affect fungal entomopathogens

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The changes in the International Code of Nomenclature for fungi, algae and plants (a new name!) adopted at the 2011 International Botanical Congress brought a mix of the good, the bad, and the ugly. Most people will welcome the ability to publish descriptions and diagnoses of new taxa in English (or Latin), and to publish new taxa in a wide range of online rather than print media. Many people, however, may regard the elimination of dual nomenclature for the conidial and sexual states of individual pleomorphic fungi (e.g., the conidial states of ascomycetes in Hypocreales—the most common and best known entomopathogenic conidial genera) to be an unfortunate step backward forced by the adoption of a new standard referred to as 'One Fungus = One Name' (IF=IN) that will accept only a single generic name in the future for all connected conidial and sexual forms of fungal genera while suppressing all other linked genera; committees will have to choose which names to accept and to suppress, and will supposedly favor the earliest published applicable (sexual or conidial) generic name. These changes in the Code will have disruptive and destabilizing effects for several years, and will affect few fungi more severely than hypocrealean entomopathogens (e.g., *Beauveria*, *Cordyceps*, *Isaria*, *Lecanicillium*, *Metarhizium*, *Nomuraea* and many more). This poster explains the changes and suggests what might be the probable–and, for many of us, unwelcomed–decisions that will probably be reached for these fungi.

Poster - Fungi Wednesday 16:45 **F-29**

Phylogenetic reclassification raises new respect–and a new phylum!–for Entomophthorales

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The recent phylogenetic studies and reclassifications produced by the global All-Fungal Tree of Life study recognized the Entomophthorales (as historically treated, with Basidiobolus remaining in this order) as a new subphylum, Entomophthoromycotina, without being placed in any phylum. Subsequent phylogenetic analyses of the broadest range of entomophthoroid taxa and more genes than in any previous studies confirm the monophyletic nature of these fungi and their distinctness from all other groups formerly classified in Zygomycota. As a lead-in to the publication of these molecular and traditional taxonomic analyses, the subphylum is now formally raised to phylum level (Entomophthoromycota), and its included fungi reclassified into three classes (Basidiobolomycetes, Neozygitomycetes, and Entomophthoro–mycetes), while two genera of the family Meristacraceae, Ballocephala and Zygnemomyces, have been removed from the Entomophthorales to the subphylum Kickxellomycotina.

Poster - Fungi Wednesday 16:45 **F-30**

Pathogenicity of *Metarhizium anisopliae* (Metchn.) Sorok on *Blattella germanica* (Linnaeus) (Blattodea: Blattellidae) and *Periplaneta fuliginosa* (Seville) (Blattodea: Blattidae) in Argentina

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Cockroaches have a worldwide distribution and survive well in association with any human settling. They are important vectors of pathogens that cause disease in animals and humans. Cockroaches are controlled primarily by synthetic organic insecticides. An alternative to chemical methods is the use of entomopathogenic fungi. The isolation *of Metharizium anisopliae* (Metschnikoff) Sorokin, (*Ma*) CEP 085 from the culture collection of entomopathogenic fungi of CEPAVE was use. This isolate originally proceed from an unidentified Hemiptera, Cercopidae from Argentina. Pathogenicity was assessed for adults and nymphs (III) of *B. germanica* and *P. fuliginosa.* The insects were exposed to conidia of *M. a*. by direct contact. The conidial suspension of $1x10^9$ spores per milliliter, was applied in Petri dishes with filter paper and cockroach were collocated for 24 hs. The treated cockroaches were placed in plastic containers, were fed with dog food and tap water. The control insects were treated with Tween 80 0.01%, under the same conditions. The bioassay was conducted at 25 ± 2ºC and 70 ± 5% RH. Mortality was controlled daily for 20 day, and dead cockroaches were removed and placed into sterile Petri dishes. The emergence of hyphae was monitored for 8 days. Adults and nymphs of *B. germanica* were susceptible to *Ma 085* infection, with mortality up to 50%. However *P. fuliginosa* mortality was lower than 25% in adults and nymphs. The differential susceptibility at Ma could be related with the composition of the cuticle in the different species.

Poster - Fungi Wednesday 16:45 **F-31**

Lipolytic and proteolytic activities of *Metarhizium anisopliae sensu lato* isolates associated to its virulence on *Rhipicephalus microplus* ticks

Wendell Marcelo de Souza Perinotto[1], Patrícia Silva Golo[1], Lucélia Santi[2], Marilene Henning Vainstein[2], Walter OrlandoBeys da Silva[2], Cristiane Martins Cardoso Salles[3] and Vânia Rita Elias Pinheiro Bittencourt[1]

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Previous studies have demonstrated that the hydrolysis of some fungal enzymes assists the fungal penetration into host. Accordingly it is necessary to select isolates potentially virulent to be used in biological control programs. This study assessed, thus, the *in vitro* lipolytic and proteolytic activity of five isolates of *M.anisopliae* s.l. and correlated these results with *in vitro* tests against *Rhipicephalus microplus* engorged females ticks. Conidia were inoculated in minimal medium (MM; 0.1 % KH2PO4 and 0.05 % MgSO4) containing 1 % *R. microplus* cuticle or 1 % glucose (G) as a control condition. The flasks were incubated at 25 °C at 150 rpm. After 24, 48 or 72 h incubation, mycelia were harvested by filtration and the culture filtrate used for the experiments. The lipolytic activity was assayed using ρ –nitrophenyl palmitate as substrate. For the protease assay, the chromogenic substrate N-suc-ala-ala-pro-phe-pNA was used. Concurrently, the *R. microplus* bioassay was performed. Engorged females were immersed in 1 ml of 10^8 conidia $^{-1}$ suspension of each isolate, for three minutes. The three isolates (CG 32, CG 148 and CG 629) that caused the highest percentage of tick mortality presented high lipolytic activity from 24 h and high proteolytic activity between 48 and 72 h. We suggested that there is association between the lipolytic and proteolytic activities and the virulence potential of *M. anisopliae* s.l. fungi on *R. microplus*, assuming that the three isolates can be used as biological controllers of this tick.

Poster - Fungi Wednesday 16:45 **F-32**

Conidial Pr1 activity of *Metarhizium anisopliae*: a comparative study of the proteolytic activity of conidia produced on artificial medium or tick cadavers

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Subtilisin-like serine proteases Pr1 belong to an important family of proteases involved in fungal infection process. The current study investigated the Pr1 proteolytic activity of *Metarhizium anisopliae* conidia produced on potato dextrose agar (PDA) medium and compared it to the Pr1 activity of conidia emerged from *Rhipicephalus microplus* ticks after induced infection. The fungus was cultivated on PDA for 14 days at 25°C and relative humidity (RH) ≥ 90%. *R. microplus* engorged females were inoculated with fungus and hold at 25°C and RH ≥ 90%, and 14 days later conidia had already exteriorized the cadaver. Conidia were harvested from PDA or removed from dead ticks by sieves stirring, and then suspended in an extraction buffer (Tris–HCl 50 mM pH 8.0 containing 0.25% Triton X-100, 1:2.5 w/v). The suspensions were shaken for 5 min and the resulting supernatants were filtered through a 0.2 mm-pore-size filter. The supernatant was used for enzymatic assays. The substrate suc-ala-ala-prophe-ρNA was tested at 0.2mM in a final volume of 100 mL. Kinetic assays were monitored at 37 °C for 30 min in a spectrophotometer equipped with thermostat and shaking systems. One protease unit (PU) was defined as the amount of enzyme that produces one ρmol of ρ-nitroaniline per hour. Proteins surface of conidia produced on artificial medium presented 4PU of proteolytic activity while proteins surface of conidia collected from infected ticks presented activity 9 times higher, 36.17PU. Pr1 proteases are involved in early stages of fungal infection and, according to our results, conidia produced over the cuticle of infected ticks increased the enzymatic activity of these proteins. This study contributes to clarify the dynamics of fungal infection in ticks.

Poster - Fungi Wednesday 16:45 **F-33**

Susceptibility of *Galleria mellonella* larvae parasitized by ectoparasitoid *Habrobracon hebetor* to anamorphic entomopathogenic ascomicetes

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The mycosis of *Galleria mellonella* larvae envenomated by ectoparasitoid *Habrobracon hebetor* was investigated. Immunosuppressive effects of ectoparasitoid venom on phenoloxidase activity in haemolymph and encapsulation response of *G.mellonella* larvae envenomated by *H. hebetor* have been shown. We found that the envenomated larvae were more susceptible to the *Metarhizium anisopliae, Isaria farinosa, I. fumosorose* and *Beauveria bassiana*. The LC_{50} for *B.bassiana* was decreased almost 5,000 times. Envenomated larvae were susceptible to 100 conidia of fungi while the non-envenomated larvae were susceptible to $5x10^5$ dose of conidia. It has been shown that mycosis occur in both envenomated (venom) and parasitized (venom and ectoparasitoid larvae) *G. mellonella* larvae.

Poster - Fungi Wednesday 16:45 **F-34**

Anti-fungal activity of protein extracts on the *Bipolaris oryzae* and *Gerlachia oryzae* phytopathogens

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This study proposes to evaluate the *in vitro* effect of vegetable extracts on the phytopathogens *Bipolaris oryzae* and *Gerlachia oryzae*. Liquid extracts of medicinal plants were obtained by maceration and then dialyzed 3kDa retention membrane. To determine the antifungal activity two methodologies were used: Kirby-Bauer and incubation. Analysing the protein profile in 15% SDS-PAGE, we observed in the *Ruta graveolens*, *Symphytum officinale*, *Tanacetum vulgare*, *Petiveria alliaceae* and *Artemisia absinthium* extract, bands representing polypeptides, with molecular mass between 50 and 30 or 20 and 10kDa. When the phytopathogenic fungi *G. oryzae* was treated with the *T. vulgare* and *A. absinthium* essences, the mycelia growth did not differ from that of the control, while the other treatments demonstrated the fungi-static action of *G. oryzae*, even after the 14° After Day Treatment (ADT). It was also observed that treatment with the *Malva* sp., *A. absinthium*, *Z. officinale* and *C. citratus* essences totally inhibited the formation of the Colonies Formation Units (CFUs). Similarly, when the *B. oryzae* fungi was submitted to the treatment with the essences, it was observed that the *T. vulgare*, *Mentha* sp. and *R. graveolens* essences did not inhibit the mycelial growth which remained unaltered until the 14° ADT. On the other hand, the remaining treatments did not differ from the control group ($p< 0.05$). This research proved that the *Z. officinale*, *C. citratus* and *Malva* sp. extracts control *G. oryzae* best while the *P. alliacea* extract is the most efficient against *B. oryzae*.

Poster - Fungi Wednesday 16:45 **F-35**

Diversity of *Metarhizium* spp. isolates from Western and Central United States

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Cadavers of soil-dwelling and terrestrial arthropods killed by entomopathogenic fungi routinely produce numerous conidia that are deposited within or on the soil. In this study, *Metarhizium* spp. isolates were cultured from approximately 17,800 soil samples collected from Western and Central West states of the United States during 2007, 2008 and 2009. This survey aims to identify, and determine genotypic and phenotypic diversity among *Metarhizium* spp. in order to search for potential biological control agents for insect-pests, particularly acridids. Initial isolations of *Metarhizium* were made using CTC selective medium, and provisional assignment to genus was based on macro- and micro-morphological characters. *Metarhizium* species were identified by using partial elongation factor 1-alpha sequences in accordance with Bischoff et al. (2009). Additionally, isolates were screened for their tolerance to heat and ultraviolet (UV-B) irradiation, for identifying those with exceptional potential to persist in the environment. A total of 32 5'-EF-1α haplotypes were detected among 489 *Metarhizium* soil isolates that included, in order of decreasing abundance: *M. robertsii* (n=369), *M. guizhouense* (n=48), *M. brunneum* (n=39), *M. lepidiotae* (n=14), *M. flavoviride* (n=11), *M. anisopliae* (n=6), or *M. pingshaense* (n=2). The isolates varied widely in thermotolerance, with conidial relative germination of isolates exposed to 45 ± 0.2ºC for 5h ranging from 0% to close to 100%. Isolates of two haplotypes were found to have exceptionally high thermotolerance; one was identified as *M. robertsii* and the other as *M. anisopliae*. In general, conidia of *Metarhizium* spp. isolates were very susceptible to the UV-B dose tested (8.90 KJ m^{-2}), with relative germination not exceeding 50%. This survey discovered novel indigenous USA Metarhizium spp. isolates from widely dispersed locations, and the new isolates are the focus of further characterization studies to screen them for novel biological control agents of pest insects.

Poster - Fungi Wednesday 16:45 **F-36**

Pathogenicity and horizontal transmission of entomopathogenic fungi to *Diaphorina citri* (Hemiptera: Psyllidae)

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The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is the most important citrus pests worldwide, because it is the vector of the proteobacteria *Candidatus* Liberibacter asiaticus the causal agent of the Huanglongbing (citrus greening disease). The objectives of this study were 1) to evaluate the pathogenicity of strains of entomopathogenic fungi to *D. citri* adults and 2) to assess contamination between sporulated cadavers and healthy adults under laboratory conditions. Bioassays were carried out with 17 isolates of *Beauveria bassiana*, *Isaria fumosorosea*, *I. amoenerosea*, *Purpureocillium lilacinus*, *Lecanicillium muscarium, Lecanicillium* spp., *Metarhizium anisopliae,* "*Singliocladium*" sp. and *Hirsutella thompsonii* at 1 x 10^7 conidia/mL. *I. fumosorosea* ESALQ-1296, *B. bassiana* ESALQ-PL63, *B. bassiana* ESALQ-1432, *Lecanicillium sp*. ESALQ-949 and *L. muscarium* ESALQ-972 caused higher mortalities and were selected for horizontal transmission assays. Twenty healthy adults were transferred to a citrus seedling containing one, three, five or ten sporulated adults inside a bottle cage and mortality was evaluated after 15 days. The median lethal concentration was around 2 sporulated insect (rate 1:10 infected:uninfected) for the isolates of *I. fumosorosea* ESALQ-1296, *B. bassiana* ESALQ-PL63, *B. bassiana* ESALQ-1432. At a rate of 1:2 (infected:uninfected) total mortality was higher to 94% for these isolates. Lower horizontal transmission was observed for *Lecanicillium sp*. ESALQ-949 and *L. muscarium* ESALQ-972. The isolates of *I. fumosorosea* and *B. bassiana* have potential to be incorporated in integrated pest management programs of *D. citri*.

Poster - Fungi Wednesday 16:45 **F-36**

Fungi associated with epizootic of hemlock woolly adelgid, *Adelgid tsugae* Annand (Hemiptera: Adelgidae)

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The hemlock woolly adelgid (HWA), *Adelgid tsugae* (Hemiptera: Adelgidae), has become is a principal pest of the North American indigenous hemlock species. Morphological and biological characteristics of HWA provide this pest advantages for success in colonization of hemlock forest. Investigation of natural factors regulating of adelgid populations is very important for biological management of pest. From 2008 to the present a natural local epizootic has been observed in population of HWA in the southern New Hampshire. Mortality of HWA was practically 100% in the center of epizooty. Pathological material from center of epizootic was used for isolation of fungi associated with mortality of insects. The three groups of fungi were isolated including entomopathogenic, phytopathogenic and epiphytic species. Entomopathogenic fungi *Myriangium* sp., *Beauveria bassiana*, *Lecanicillium* sp. and *Phoma* sp. were isolated. The fungus *Phoma* sp. from phytopathogenic genus was included in the entomopathogenic group because his propagules were presented directly on the insect bodies of HWA. Besides, there is the information about entomopathogenic fungus *Phoma aspidioticola* attacking the armored scale, *Aspidiotus destructor*, in India (Narendra and Rao, 1974). On the basis of the lead researches it is possible to draw a conclusion, that the reason of mass mortality of insects is activity of specialized entomopathogenic fungus *Myriangium* sp. Fungus *Phoma* sp. can be involved in epizootiological process.

POSTER SESSION 2 Wednesday, 16:45 – 18:45

MICROBIAL CONTROL

Poster – Microbial Control Wednesday 16:45 **MC-24**

Microbial control of *Pseudoplusia includens* (Walker) and *Anticarsia gemmatalis* Hübner with their viruses, PsiSNPV and AgMNPV

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In Brazil, populations of soybean looper [*Pseudoplusia includens* (Walker)] have become more important since the early 2000s, possibly due to fungicide widespread use against soybean rust. Fungicide applications suppress important entomopathogenic fungi, such as *Nomuraea rileyi*, *Pandora gammae*, *Zoophtora radicans*, and *Isaria tenuipes*, which probably favors insect population growth. Usually, soybean looper and velvetbean caterpillar (*Anticarsia gemmatalis* Hubner) infestations occur

simultaneously. To verify the efficiency of the simultaneous application of viruses that attack both species, we conducted two experiments, one in 2010/2011 and another in 2011/2012. In the first field assay, we applied the following virus rates (occlusion bodies - OB.ha^{-1}): 1) Control; 2) PsiSNPV = 1×10^{12}; 3) AgMNPV = 1.5×10^{11}; 4) PsiSNPV=1×10^{12} + AgMNPV = 1.5×10^{11}; 5) PsiSNPV = 0.5×10^{12}; 6) AgMNPV = 0.75×10^{11}; 7) PsiSNPV = 0.5×10^{12} + AgMNPV = 0.75×10^{11}. In the second field assay, we applied the following virus rates (OB.ha^{-1}): 1) Control; 2) PsiSNPV = 2×10^{12}; 3) PsiSNPV = 3×10^{12}; 4) PsiSNPV = 2×10^{12} + AgMNPV = 1.5×10^{11}; 5) AgMNPV = 1.5×10^{11}; 6) PsiSNPV = 3×10^{12} + AgMNPV = 1.5×10^{11}; 7) AgMNPV = 0.75×10^{11}. In the first experiment, the viruses did not control soybean looper and velvetbean caterpillar field populations even using 1×10^{12} and 1.5×10^{11} OB.ha^{-1}, respectively. In the second experiment, soybean loopers were prevalent, and virus treatment (2×10^{12} and 3×10^{12} OB.ha^{-1}) caused a slightly reduction in the number of individuals larger than 2.5cm. No additive or synergistic effects were observed in laboratory bioassays or in the field. No control of small larvae (<2.5cm) was observed, possibly due to continuous oviposition throughout the soybean season. In plots treated with PsiSNPV, during 18 days of evaluation, the defoliation ranged from 17% to 20%, whereas in the control plots it ranged from 20% to 22%.

Poster – Microbial Control Wednesday 16:45 **MC-25**

Evaluation of *Cydia pomonella* granulovirus (CpGV) in combination with Rynaxypyr and Metoxyphenozide for codling moth control in walnuts orchards in Catamarca, Argentina

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Traditionally, codling moth (CM) control in the main walnut growth area was based on the use of broad spectrum insecticides (Azinphosmethyl, Fhosmet or Lambda-Cyhalothrin). Alternatively, a CpGV based-product (CARPOVIRUSplus®) has been successfully used against this pest as unique tool or in combination with such insecticides. With the aim to find more friendly environmental practices, two selective insecticides, Rynaxypyr (Coragen®) and Methoxyfenozide (Intrepid SC®), had been evaluated in combination strategies with the virus. Treatments of CpGV were compared with alternations of virus with chemical insecticides: CpGV followed by Rynaxypy or Methoxyfenozide against 1st and 2nd flights, respectively and viceversa. CpGV treatments showed no differences compared with combined treatments neither when rynaxypyr or methoxyphenozide were sprayed against first larval generation plus CpGV nor when they were applied on 2nd generation. In the scheme CpGV followed by Rynaxypyr or methoxyfenozide, damage levels at harvest ranged from 0.35 to 0.5 respectively, versus 0.6 in CpGV treatments . When Rynaxypyr and methoxyfenozide were sprayed before the virus over s over 2nd flight, damage levels showed values between 0.4 and 0.6, respectively. All treatments aroused differences compared with up to 47% injury registered in the untreated plots. In conclusion, the use of CpGV alone offered a success control similar to those achieved when was combined with rynaxypyr or methoxyfenozide under field trials conditions. The incorporation of these two selective insecticides into integrated pest management (IPM) of CM is promissory in order to avoid or delay the expression of resistance to CpGV with a minimum environmental impact.

Poster – Microbial Control Wednesday 16:45 **MC-26**

Effect of *Bacillus thuringiensis* on the phytophagous activity of *Podisus nigrispinus* on kale leaves and on its consumption of *Plutella xylostella* larvae

Alessandra Marieli Vacari, Gustavo Oliveira de Magalhães, Valeria Lucas de Laurentis, Haroldo Xavier Linhares Volpe, Ana Carolina Pires Veiga, Sergio Antonio De Bortoli and Ricardo Antonio Polanczyk

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The objective of this study was to evaluate the effect of *Bacillus thuringiensis* on the phytophagous activity and prey consumption of *Podisus nigrispinus*. *Plutella xylostella* larvae were offered as prey to the predators. The leaves were exposed to the predators and were replaced every 2 days; the leaves that were removed were subjected to immersion in 1% acid fuchsin for 12 hours. They were then washed with water in order to count the food sheaths with the aid of a stereomicroscope. A total of 3 treatments were used: treatment with *B. thuringiensis*-infected larvae, which involved the HD1 strain (3 × 10^{8} spores/mL); treatment with *B. thuringiensis*-infected larvae, which involved Agree®, a commercial product (0.5 g/333 mL recommended for kale); and the control treatment, which involved water. Overall, consumption during the nymphal period differed among the infected larvae treatments and the control; the consumption was higher for Agree® and HD1 (19.4 and 17.9 prey, respectively) than for the control (14.6 prey). During adulthood, the number of times that predators placed the stylus on kale leaves was similar for leaves treated with water (341.5 food sheaths) and for those treated with the isolate HD1 (411.5 food sheaths), but low levels of phytophagy were observed for leaves treated with Agree® (218.8 food sheaths). The results showed that application of Agree® on plants favored prey consumption and impaired the phytophagous activity of *P. nigrispinus* on kale leaves. Thus, this product can contribute to the agricultural ecosystem balance and to production of food that is safe for human consumption.

Poster – Microbial Control Wednesday 16:45 **MC-27**

Efficacy of an aqueous suspension of *Bacillus thuringiensis* var. *israelensis* against *Aedes vexans* larvae in Xinjiang Irtysh river lower reach area

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The research was carried out at Beitun town, located on the basin area of Irtysh river lower reach, in Altay District, Xinjiang Province, northwest of China. The density of immature mosquitoes in all kinds of pools was investigated from May to August. An abundance of larvae was observed in the pondings caused by sluicing to irrigate meadow, the ditches to eliminate alkalinity all over the farm and the forest belt forest blet which was flood irrigating. The curves of seasonal distribution of the larvae were two peaks type. The peak appeared in 23 May to 10 June and 25 June to 10 July. Morphology identification had been done to the mosquito samples collected in outskirts of Beitun, *Aedes vexans* is dominant species. The efficacy of aqueous suspension of *Bacillus thuringiensis* var. *israelensis* was tested against the *A. vexans* larvae in the laboratory. The bioassays tests showed that the LC50 and LC90 values of aqueous suspension being 0.055 and 0.198 ppm. Open field test were conducted in three high density habitats, with the application of formulations at 2 ppm, more than 96% reduction of larvae was observed for 48 hours, minimum effective dosages to achieve elimination of the larval population in a given habitat are extremely low and environmental impact is negligible. A total of 7.4 ton formulations had been used in about 2 km^2 habitat. Adult mosquitoes decreased 50% compared with last year. We conclude that the mosquitoes in our study area are highly susceptible to those microbial control agents. Therefore microbial products for larval control have great potential in Xingjiang province.

Poster – Microbial Control Wednesday 16:45 **MC-28**

Leaf consumption of *Plutella xylostella* assayed with *Bacillus thuringiensis*

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Plutella xylostella is the most important pest of Brassicaceae plants and the insecticides used against this pest can cause undesirable effects on the environment and residues on the food. *Bacillus thuringiensis* (*Bt*) is the most important bioinsecticide worldwide and usually highly virulent to *P. xylostella*. To demonstrate the effect of *Bt* on *P. xylostella* leaf consumption and possible differences between pest populations, 150 second instar larvae from four Brazilian populations named PC (44th generation), PA (41th generation), PX (74th generation) and PJ (2th generation) were assayed at laboratory with bioinsecticides (Xentari®, Agree®, and Dipel®) and 11 isolates (E47, HD-1, 49.19, T08, E28, 41.7, BTT090, 20.7, E7, *B. thuringiensis tenebrionis,* and 153.3). The average leaf consumption of the control was 41.28 cm^2 and higher than that verified in a second group of treatments: 20.7, BTT090 and *B. thuringiensis tenebrionis*. Furthermore the second group consumption was higher than for the isolates 41.7, 153.3, E7, T08 and E28. Evaluating leaf consumption was not possible for all bioinsecticides and the isolates E28, E47, HD-1 and 49.19 because all larvae died in bioassay. Among populations, in general the higher leaf consumption submitted to *Bt* occurred to PA and PC populations and the lower one was verified to SBT and PX populations. Leaf consumption is an important parameter that can contribute to the background around the interaction between *P. xylostella* and *Bt*.

Poster – Microbial Control Wednesday 16:45 **MC-29**

Encapsulation of *Bacillus thuringiesis* Vip3A toxin in *Pseudomonas fluorescens* as a way to develop new spray bioinsecticides

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Vip3 toxins, secreted during the vegetative growth of *Bacillus thuringiensis*, do not share homology to the crystal (Cry) proteins and are active against several species of Lepidoptera. Due to its toxic potential and different mode of action, Vip3A proteins have been combined with Cry1Ab in transgenic crops. However, since Vip3 toxins are released from the cell during fermentation, they are excluded from *B. thuringiensis* spray formulations, which are based on a mixture of spores and crystals. As an approach to obtain a novel bioinsecticide containing Vip3 toxins, in this study Vip3Aa1 has been expressed in *Pseudomonas fluorescens*. This bacterium, without pathogenicity to animals or plants, can be used as a biological agent after a killing-fixation process that strengthens the *P. fluorescens* cell wall. This encapsulation process has been shown to protect Cry proteins from UV light and environmental factors. Vip3Aa1 expression into *P. fluorescens* was achieved by the cloning of the *vip3Aa1* gene into the broad range expression vector pMEKm12. Inducible expression of Vip3Aa1 from pMEKm12 was observed in both *E. coli* and *P. fluorescens* by SDS-PAGE and Western Blot. Proteolysis of Vip3Aa1 with trypsin or *Spodoptera frugiperda* midgut juice rendered the expected digestion fragments. The toxicity of Vip3Aa1 expressed in *P. fluorescens* was tested against *S. frugiperda* and the LC_{50} was 89 ng/cm^2, a value similar to that obtained when this protein is expressed in *E. coli*. The results support the suitability of *P. fluorescens* as a delivery vector for the development of bioinsecticides based on Vip3A toxins from *B. thuringiensis*.

Poster – Microbial Control Wednesday 16:45 **MC-30**

Hemicellulose compatibility to *Beauveria bassiana* and *Metarhizium anisopliae* fungi and their effect on development parameters of the entomopathogens

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The sugarcane borer (*Diatraea saccharalis)* and sugarcane weevil (*Sphenophorus levis*) are high-important pests in the cultivation of this crop. The use of entomopathogenic fungi for pest control is a fact in Brazil; however, one of the greatest challenges for increasing it is the lack of formulated products whose stability lasts for long periods at room temperature. The conidia microencapsulation by biodegradable polymers is a promising technique for providing protection and extending conidia storage. In this work, the hemicelluloses - found in vegetables - was extracted from sugarcane bagasse. The objective of this study was to evaluate the hemicellulose compatibility to the entomopathogenic fungi, as well as calling attention to its use in the microencapsulation process. IBCB 66 (*B. bassiana*) and IBCB 45 (*M. anisopliae*) isolates were used and tested at concentrations of 0.1%, 0.5%, 1% and 2% of hemicellulose added to the PDA culture medium before its solidification at 45 °C. The control was done only using PDA. The effects of the biopolymer were determined by evaluating the fungi vegetative growth and sporulation after 7 days of culture under growth chamber at 26.5 °C, and its viability after 24 hours of incubation. Data were analysed by Tukey test. Toxicity was evaluated by the Biological Indicator formula (BI), developed by Alves *et al*.(2007)*. Hemicellulose presence didn´t affect the fungal growth. In the BI analysis, the hemicellulose concentrations tested were compatible to the fungi showing a promissory biopolymer when used in the microencapsulation process.

Poster – Microbial Control Wednesday 16:45 **MC-31**

Determination of Lethal Concentration 50 of Beauveria *bassiana* and *Metarhizium anisopliae* fungi to the sugarcane borer *Diatraea saccharalis*

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The sugarcane borer (*Diatraea saccharalis*) is one of the main pests which attack the sugarcane crop and may also causes it direct and indirect damages. The fungi *Beauveria bassiana* and *Metarhizium anisopliae* are proved pathogenic to that pest, being found and used for controlling other sugarcane pests in Brazil. However, most of the used fungal products aren´t formulated, which turns its application and shelf life into difficult processes. Based on that, this work aims to determine the lethal concentration 50 (LC_{50}) to those two fungi in order to develop a microencapsulated product. IBCB 66 (*B. bassiana*) and IBCB 425 (*M. anisopliae*) isolates were used as well as the concentrations 1x10^8, 5x10^8, 1x10^9 and 5x10^9 conidia / mL were tested. The *D. saccharalis* caterpillars were immersed in 20 mL of standardized fungal suspensions of each fungus for one minute. The control group was only immersed in distilled water. After inoculation, six caterpillars were placed in each container. The experiment was tried out for 5 times and it resulted in 30 insects/treatment. The containers were kept in controlled temperature rooms at 25°C. After 24 hours of inoculation, a piece of sugarcane / container was offered to feed the caterpillars. The mortality evaluations occurred daily – until 7th day – and humid chambers were used to confirm the caterpillar's mortality by the fungi. The LC_{50} was calculated by Probit analysis: 1,93x10^7 conidia / mL for *B. bassiana*, and 3,10x10^8 conidia / mL for *M. anisopliae*.

Poster – Microbial Control Wednesday 16:45 **MC-32**

Effect of formulation on the oily conidial viability of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes)

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The dynamics of technological innovation enables the development of bioproducts containing entomopathogenic fungi such as *Beauveria bassiana* and *Metharizium anisopliae*. The objective of this research was evaluating the effect of oily vegetable and mineral formulations on conidia of *B. bassiana*. The *B. bassiana* was produced on semi-solid rice conidia were separated by sieving and dried for 72 hours. The fungus (0.4 g) was mixed with the formulations according to the order of each treatment (T): T1 (T80 + fungus + oil), T2 (T80 + oil + fungus), T3 (T80 + S80 + oil + fungus), T4 (T80 + S80 + oil + fungus), T5 (+ fungus + kerosene oil), T6 (T80 + fungus + kerosene oil), T7 (kerosene + oil + T80 + S80 + fungus), for both types oil. The formulations were stored in a chamber at 26 °C. The control group comprised samples of pure conidia and rice + fungus, BOD and stored in freezer, all for a period of 12 months. In the third step, every 30 days, the formulations and the control group were evaluated by testing the feasibility and the end of the experiment through an analysis of contrasts. It was found that better preserve oily formulated conidia at room temperature showing percentage of conservation very close to controls stored in the freezer for a period of 180 days. The analysis of contrasts showed a significant difference for formulations with and without kerosene.

Poster – Microbial Control Wednesday 16:45 **MC-33**

Formulation of entomopathogenic fungus, *Beauveria bassiana* (Vuill.) in alginate matrix

Ísis Tatiana Borges Jordão Braga[1], Marcelo da Costa Mendoça [2] and Ana Amélia Moreira Lira[3]

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The fungus *Beauveria bassiana* has the longevity of their conidia reduced when stored at ambient conditions alone. The aim of this study was to develop formulations containing conidia of *B. bassiana* allowing its preservation. The fungus was produced on semi-solid rice. The rice and the fungus was dried in air conditioned environment at 18°C ± 2°C, 20% RH and after 3 to 5 days sieved to obtain the pure conidia. After this process, pure conidia showed 91.8% viability and 6% moisture. Spores were immobilized in alginate matrix by ionotropic gelation technique forming two treatments (formulations): a) soy oil + yeast + 4% alginate, b) soybean oil + yeast + 5% alginate, in four replicates, plus the control (pure conidia). After six months of storage under refrigeration (4°C and 50% RH) the formulations containing 4% and 5% alginate had a survival rate of 81.4% and 77.7% respectively. After this evaluation, the formulations with alginate and 5% to 4% and pure conidia were transferred to BOD temperature (26°C±2°C) with 30% RH, showing the viability of 57.07%, 44.13% and 58.41% respectively, after one month of storage.

Poster – Microbial Control Wednesday 16:45 **MC-34**

Management of *Meloidogyne enterolobii* in culture of guava, in Brazilian semiarid region, with the fungi *Paecilomyces lilacinus* and *Trichoderma* spp.

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The expansion of the guava cultivation (*Psidium guajava*) in the Brazilian semiarid region has been limited by the presence of the nematode *Meloidogyne enterolobii*. Studies indicate the efficacy of *P. lilacinus* and *Trichoderma* spp. on control of nematodes of the genus *Meloidogyne*. The aim of this study was to evaluate the efficiency of the products ICB NUTRISOLO Paecilomyces (PAE) and ICB NUTRISOLO Trichoderma (TRIC), in reduction of M. enterolobii in guava orchard, in Brazilian semiarid region. The experiment was conducted in the city of Petrolina – Pernambuco - Brazil in commercial guava orchard, cv. Paluma, two years old, 6x4 m spacing, drip irrigated, with previous history of high infestation of *M. enterolobii* in the area. The products were administered monthly by irrigation system at a dosage of $5x10^{11}$ CFU/plant of the product PAE and $5x10^{10}$ CFU/plant was TRIC product. The evaluation of the efficiency of the products took place through quantification of the nematodes from soil samples of 80 plants, at three different times. With the treatment application, the *M. enterolobii* population decreased the nematode number in plant sampled at 77.5% and the control level in these plants varied from 51.72 to 96.93%. Thus, it can be seen that the tested products have proved agronomical effectiveness for control of *M. enterolobii* in guava tree.

Poster – Microbial Control Wednesday 16:45 **MC-35**

Molluscicidal activity of *Bacillus thuringiensis* against golden mussel, *Limnoperna fortunei*

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Golden mussel, *Limnoperna fortunei* is an Asian bivalve mollusc, probably introduced in Brazil through ballast water. This species could be a great impact on indigenous species and in water catchment or energy generation areas causes losses, due to formation of clusters in equipment. Physical and chemistry methods of control are being evaluated, but there isn`t report of pathogens for this species. This study aimed evaluate to efficiency of products based on *B. thuringiensis* sv. i*sraelensis* in golden mussel control. Two formulations of VECTOBAC product (Sumitomo Chemicals): aqueous (AS) and water granules (WG) were evaluated. Bioassays were conducted in beakers containing 250 mL water reconstituted and ten adults *L. fortune.* Three different concentrations of the products (0.5, 1 and 2 mL) were tested and done in quintuplicate. The assay was conducted at 21 ±2 °C with oxygenation provided by aerators. Controls were done by maintenance of mussels in water reconstituted. Mortality was assessed daily during seven days. The higher mortality (86%) were obtained with 2 ml of the VECTOBAC AS product concentration, followed by 55% with 1 mL and 20% with 0,5 mL of the product. The granular formulation was not effective in controlling the pest. This is the first report of using a commercial microbial product for golden mussel control.

Poster – Microbial Control Wednesday 16:45 **MC-36**

Identification of serpins in hemolymph of *Rhipicephalus microplus* infected by entomopathogenic fungi

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Serpins are involved in the defense mechanism of arthropods. This study analyzed the free-cell hemolymph proteins involved in immediate immune defense in *Rhipicephalus microplus* engorged females challenged with *Metarhizium anisopliae* or *Beauveria bassiana*. Three groups of 90 ticks each were inoculated with conidial suspension; in control group, ticks were inoculated with control solution (sterile distillated water and Tween 80 0.1% v/v). Hemolymph was collected from the dorsal surface of engorged females 24 hours after treatment. The cell-free hemolymph was separated from hemocytes by centrifugation and cell-free hemolymph samples were stored at -80°C with 30µL protease inhibitors cocktail and 82µL saline buffer (1.5M NaCl, 50mM EDTA, phenylthiurea). Cell-free hemolymph total protein amounts were determined using the Lowry modified method. The proteome of cell-free hemolymph was evaluated by 2D-PAGE. Hemolymph sample volumes were adjusted in order to analyze the same amount of protein (100 µg). The spots were submitted to digestion proteins with tripsin and then analyzed using proteomics approaches. Proteins were identified by matrix-assisted laser desorption and ionization tandem mass spectrometry (Maldi-Tof-Tof). The results showed *B. bassiana* infection increased the production/release of serpins in cell-free hemolymph, while the inoculation of control solution or *M. anospliae* suspension did not cause production/release of serpins. The results suggested *M. anisopliae* inhibits the immune response of *R. microplus* by curbing the serpins production/release. Further studies are necessary to clarify the immune response of *R. microplus* ticks after infection by entomopathogenic fungi, what contributes to the development well succeed control strategies of ticks.

Poster – Microbial Control Wednesday 16:45 **MC-37**

Comparative study between formulations of entomopathogenic nematode-infected cadavers to control *Rhipicephalus microplus* ticks

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Entomopathogenic nematodes (EPNs) may be applied to control pests through formulated nematode-infected insect cadavers, in which infected insects are released into the soil as a source of EPNs. After a few days, the EPNs leave the insect cadaver and seek new hosts to infect, controlling the target pest. The current study aimed to compare the effectiveness of different host cadavers to control the cattle tick *Rhipicephalus microplus*, using *Galleria mellonella* or *Tenebrio mollitor* as nematode-infected insect cadavers. Two nematode-infected insect cadavers were placed in plastic pots (300 mL) containing 150g of soil. After one week, five *R. microplus* engorged females were added to these plastic pots. Five groups of ten repetitions were tested: two groups were exposed to *Heterorhabditis bacteriophora* HP88 or *H. indica* LPP1 formulated in *G. mellonella* cadavers; other two groups were exposed to the same nematodes formulated in *T. mollitor* cadavers; and in the control group, ticks were placed under the same conditions but without EPNs and insect cadavers. The plastic pots were maintained at 27 ± 1°C and 80 ± 10% relative humidity. In the groups exposed to *H. bacteriophora* or *H. indica* formulated in *G. mellonella* cadavers there was 99.9% of tick control, while in the groups exposed to *H. bacteriophora* or *H. indica* formulated in *T. mollitor* cadavers there was, respectively, 76.9% and 78.6% of tick control, suggesting that EPNs formulated in *G. mellonella* cadavers are more effective to control *R. microplus*.

Poster – Microbial Control Wednesday 16:45 **MC-38**

Advances in the research about mycoacaricides against RMSF vectors in Latin America

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Tick control with mycoacaricides can be improved with virulent fungal species and specific formulation and application techniques. A quick elimination of gravid females and their eggs in non-host environments with naturally occurring or artificially released pathogenic fungi will reduce the number of new potential tick vectors. An 18-month survey of fungi in field-collected *Amblyomma cajennense*, an important vector of Rocky Mountain spotted fever (RMSF) in Latin America, and of their habitats confirmed *Beauveria bassiana*, *Purpureocillium* (*Paecilomyces*) *lilacinum* and *Metarhizium anisopliae* as natural antagonists of this tick in Central Brazil. Oil-in-water formulated *M. anisopliae* conidia applied directly on eggs or after exposure of eggs on treated filter paper, inhibited best larval eclosion of *A. cajennense* and *Rhipicephalus sanguineus*, another potential RMSF vector. After mixing conidia with soil, *M. anisopliae* recycled on small organic particles, and no larvae eclosed regardless of the conidial titer applied. Gravid *A. cajennense* exposed to oil-formulated *M. anisopliae* conidia under semi-field conditions in 2011 in the early dry season (April) in Central Brazil initiated oviposition, and thereafter mycelium and conidia developed on the eggs; eclosion did not occur at all or was reduced after increasing time between application and exposure of gravid females. *Metarhizium* sp was detected on treated substrates for one month after application. Findings strengthened the potential of oil-formulated *M. anisopliae* to control non-host tick stages, but also the need for more field tests and to assess such crucial issues as focal application techniques and persistence of improved fungal formulations.

POSTER SESSION 2 Wednesday, 16:45 – 18:45

MICROSPORIDIA

Poster – Microsporidia Wednesday 16:45 **M-01**

Spread of *Nosema lymantriae* in experimental gypsy moth populations – first results

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We established three 1ha exprimental sites near Bojna (Slovakia) to study the spread of *Nosema lymantriae*, a pathogen of the gypsy moth, *Lymantria dispar*. At each site, 121 *Quercus petreae* trees (60-70 years old) were marked, mapped and banded with burlap bands in early spring. Two *L. dispar* egg

masses per marked tree were released at the same time. When *L. dispar* larvae were in third instars at study sites, about 10,000 newly molted *Nosema*-infected third instar larvae were released at one or two different points. Larvae were re-collected from each marked tree and site at 10, 20 and 30 days after release (dar).

Between 49 and 174 larvae were re-collected from each site and sampling date; in total 690 larvae. Six (8.8%) and eight (16.3%) heavily infected larvae were re-collected at 10dar, indicating the survival of initially infected gypsy moth larvae. These larvae were found up to 60m away from the releasing points. While the number of recollected larvae increased, the proportion of infected larvae decreased insignificantly at 20 and 30dar. When infected larvae had been released at one point, 3.6% of the re-collected larvae acquired an infection with *N. lymantriae* 20 or 30dar and were found on average 7m and 17m away from the releasing point, respectively. When infected larvae had been released at two points, 10.1% of the recollected larvae were infected. These larvae were found up to 36m away from where *Nosema*-infected larvae had been released initially. The results will be compared with the outcome of simulation experiments.

Poster – Microsporidia Wednesday 16:45 **M-02**

Effects of *Bacillus thuringiensis* on a co-occurring microsporidian infection in *Lymantria dispar*

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The gypsy moth, *Lymantria dispar* L. (Lepidoptera, Lymantriinae) is host for a variety of pathogens, such as several microsporidian species. Outbreaks of *L. dispar* occur regularly in European oak forests; aerial spraying of *Bacillus thuringiensis* var. *kurstaki (Btk)* preparations is frequently employed in their control. We hypothesized that the combined use of *N. lymantriae* and *B. thuringiensis* causes higher larval mortality and negatively affects reproduction of *N. lymantriae*.

An LD_{50} of 1.5 IU or 1.9 IU *Btk*, respectively, was measured when 2nd or 3rd instars were infected with *Btk*. In mixed infections, a high proportion of the *L. dispar* larvae died due to microsporidiosis when *Btk* was applied in sublethal dosage. Between 87.5% and 34.2% of the dead larvae were negative for *Btk*. When low dosages of *Btk* in combination with *N. lymantriae* were fed to 2nd instars, a higher percentage of *L. dispar* larvae survived and were also free from a micosporidian infection. *Nosema*-infected cadavers of *L. dispar* contained on average $1.9*10^6$ spores/mg fresh weight (FW). This number reduced insignificantly to $1.5*10^6$ spores/mg FW and significantly to less than $0.6*10^6$ spores/mg when Btk was fed in sublethal or lethal dosages, respectively; larvae died before a massive reproduction of *N. lymantriae* took place. When *Btk* was fed in lethal dosage to, *Nosema*-infected larvae died at 4 dpi. When sublethal dosages of *Btk* were fed to *L. dispar* larvae in addition to microsporidian spores, the median time to death was not lower than in the *Nosema*-only group.

Poster – Microsporidia Wednesday 16:45 **M-03**

¨Cotton shrimp¨ disease in the freshwater shrimp *Palaemonetes argentinus* from La Plata, Argentina

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Palaemonetes argentinensis is one of the most common palaemonidae shrimp in the freshwater environmental of Argentina and since many years ago has been studied for parasites and pathogens. In a temporary pond from La Plata we found specimens with microsporidian. Infected shrimp showed a whitish coloration with the typical “cotton shrimp” aspect. The infection was recorded as spore masses embedded in the skeletal muscle. The microsporidian were studied using Interferential Contrast Microscope and TEM. Sporophorous vesicles filled with a large number of mature spores. Unfixed spores measured 4.4±0.3 (4.02–4.99 µm) × 2.9±0.2µm (2.52–3.33 µm) in size. Under TEM spores were pyriform in shape. The polar tube consists of a straight shaft and a coiled region (11–14 coils) arranged in many rows along the inside periphery of the spore. The polaroplast consisted of an anterior region of closely and loosely packed membranes. According with this features the microsporidian found in *P. argentinus* seem to belong to *Pleistophora*. More studies of the developmental stages will be necessary for the final identification. This is the second microsporidian reported from decapods crustaceans in Argentina.

Poster – Microsporidia Wednesday 16:45 **M-04**

The release and establishment of microsporidia for the biological control of *Lymantria dispar* L. in Bulgaria - results of a long-term monitoring

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The gypsy moth, *Lymantria dispar* L., is one of the most important pests of broadleaf forests worldwide, causing immense economic and ecological damage. Microsporidia are natural enemies of *L. dispar* which cause epizootics in host populations and reduce populations and damage levels significantly. In 2008, two species of microsporidia (*Nosema lymantriae, Vairimorpha disparis)* were experimentally introduced into *L. dispar* populations in the region of State Forestries Karlovo and Svoge in Bulgaria through the release of infected third-instar host larvae. The establishment and spread of the microsporidia in the *L. dispar* populations was monitored. After the introduction 2008, the microsporidium *V. disparis* was not recovered in 2009, and in 2010 only 4.3 % of the collected larvae were infected. The establishment of *N. lymantriae* was more successful: Four months after the introduction (2008), 54,8 % of all larvae collected in Karlanovo were infected (2009: 8.1; 2010: 10,3; 2011: 7,4 %). A possible reason for this difference is the tissue specificity of the two microsporidian species and their mode of transmission: While *N. lymantriae* infects the silk glands and the ovaries of the host and is transmitted perorally and transovarially, *V. disparis* is located in the fat body and transmission of spores is only possible through cannibalism or after the decay of the dead host. Since the time of introduction, no outbreak of *L. dispar* in the release areas was recorded. None of the non-target lepidopteran larvae collected on the experimental plots in 2008-2011 were infected with microsporidia, demonstrating the pathogen´s specificity.

POSTER SESSION 2 Wednesday, 16:45 – 18:45

NEMATODES

Poster – Nematodes Wednesday 16:45 **N-01**

Epizootiology of the parasite *Strelkovimermis spiculatus* (Nematoda: Mermithidae) in wild mosquito populations in Argentina

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Strelkovimermis spiculatus (Poinar & Camino, 1986) is a nematode parasite of mosquitoes isolated from the Neotropical

region. In this study we investigated the bionomics and prevalence of this parasite, in wild-mosquito populations to a better understanding of the dynamics of this nematode in temporary breeding sites. Five grassy-pool habitats filled by rainwater were sampled during a year. Eight mosquito species were collected throughout all seasons: *Anopheles albitarsis, Culex chidesteri, Culex dolosus, Culex maxi, Ochlerotatus albifasciatus, Psorophora ciliata, Psorophora cyanescens*, and *Psorophora albigenu.* Six of these species were parasitized by *S. spiculatus*: *C. chidesteri,* C*. dolosus, C. maxi, O. albifasciatus, P. ciliata,* and *P. cyanescens.* This mermithid was more frequent from the end of winter (August) to the end of the spring (November). The parasitism ranged from 11 to 100%. High levels of infections were registered only in *O. albifasciatus* larvae. This species was the most abundant with the 95% of the total mosquito larvae sampled, followed by *C. dolosus.* Infectivity of other species did not exceed 1%. *Strelkovimermis spiculatus* completed its development in all infected wild-mosquito larvae. The presence of this nematode in six natural mosquito populations increases the number of susceptible species under natural and laboratory conditions to 24. The ability of this mermithid to infect mosquito species that breed in these habitats when they remain flooded for extensive periods of time, may point to a strategy of this parasite for maintain itself for a long time in environments that periodically dry up partially.

Poster – Nematodes Wednesday 16:45 **N-02**

Persistence of *Heterorhabditis amazonenis* (Rhabditida: Heterorhabditidae) in citrus field and its virulence against *Ceratitis capitata* (Diptera: Tephritidae) *

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Ceratitis capitata is an important pest of citrus crop in São Paulo State, Brazil. This insect remains underground during its pre-pupa and pupa stages, becoming a possible target for entomopathogenic nematodes. The persistence of *Heterorhabditis amazonensis* IBCB n24 in citrus field and its virulence against *C. capitata* were assessed. Three treatments were considered: *H. amazonensis* applied at doses of 10^8 IJs/ha and 10^9 IJs/ha, and control (water). For each treatment, four replications were considered with a citrus plant per replication. Nematode persistence was based on *G. mellonella* mortality after their exposing as larvae to the treated soil. In this way, soil samples were taken before application as well as 6, 20, 33 and 49 days later. Virulence of the nematode against *C. capitata* was based on the fruit fly mortality after its exposing as pre-pupa stage to the treated soil. In this way, ten cages made of plastic screen were partially filled with soil (100 g) and partially buried on the plots at randomly distribution. Ten pre-pupae of *C. capitata* were released inside each cage, after which, the cages were closed with staples. This process was done before application as well as just after, 15, 30 and 45 days later. Concerning the nematode persistence, *H.* amazonensis provided 33 and 77% *G. mellonella* mortality for the lower and higher dose, respectively, 49 days after the application. Concerning the virulence against *C. capitata*, the nematode provided 22 and 65% mortality in the first pre-pupa releasing just after application for the lower and higher doses, respectively. Meanwhile, at the following pre-pupa releasing, the *C. capitata* mortality rates dropped and remained below 24%.

*Supported by FINEP, FAPESP, CNPq, Citrovita Company and BioControle Company

Poster – Nematodes Wednesday 16:45 **N-03**

New observations of a Trematode species in the invasive slug *Arion vulgaris*

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The invasive slug species *Arion vulgaris* (also known as *A. lusitanicus*) was first reported in Norway in 1988. Since then it has spread successfully in many parts of the country thriving mainly in coastal areas being reported as far north as Bodø (just north of the arctic circle). Most studies so far have emphasized research on managing slug populations and on studying its life-cycle. Recent studies on internal slug parasites indicate a frequent presence of nematode parasites and discovery of a trematode species in the genera *Brachylaimus*. During 2011 we collected *A. vulgaris* slugs monthly (April to November) from a locality where the presence of this trematode species in slugs had previously been observed. Slugs were dissected and the presence and number of sporocysts/metacercariae were noted. We present results from this preliminary study and discuss further work necessary to reveal the lifecycle of this trematode species.

Poster – Nematodes Wednesday 16:45 **N-04**

Endemic entomopathogenic nematodes against selected fruit fly species (Diptera: Tephridae) in laboratory studies in Tanzania

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Fruit flies (Diptera: Tephritidae) are among the most important insect pests in fruit production in many countries including Tanzania, species such as *Bactrocera* spp., *Ceratitis* spp. and *Dacus* spp.can significantly reduce yield and fruit quality. Managing damaging fruit fly populations is challenging and includes cultural control measures such as collecting and destroying infected host fruits, regulatory control (quarantine) and the use of insecticide baits. A good approach for control of fruit flies is to develop an IPM system that includes several strategies to reduce a build up of damaging pest populations. Biological control agents such as entomopathogenic nematodes (EPN) are potential candidates within such a system. In this study we isolated naturally occurring EPN in the genera *Steinernema* and *Heterorhabditis* from the Morogoro region in Tanzania. Selected EPN isolates were tested against larval and pupal stages of three important fruit fly species, *Bactrocera invadence*, *B. cucurbitae* and *Dacus bivittatus*. Results showed promise, where EPN caused 70% to 90% mortality of larvae for all three fruit fly species. Interestingly native fruit fly species appeared less susceptible to the endemic nematode species.

Poster – Nematodes Wednesday 16:45 **N-05**

Steinernema spp. infection decisions change when exposed to potential hosts infected with entomopathogenic fungi

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Entomopathogenic nematodes are exposed to a range of potential hosts in the soil. It is likely that IJs encounter hosts that have been exposed to other pathogens such as entomopathogenic fungi. The purpose of these assays was to assess the infection decisions made by entomopathogenic

nematodes (*Steinernema* spp.) in host choice assays, focusing on hosts that had been previously exposed to an entomopathogenic fungus (*Beauveria bassiana* or *Metharhizium anisopilae*). In each assay, 50 IJs were exposed in sand arenas to two waxworms: either two previously uninfected waxworms, one uninfected and one *M. anisopilae* exposed, one uninfected and one *B. bassiana* exposed, or one *M. anisopilae* exposed and one *B. bassiana* exposed. In the case of *S. glaseri*, IJs preferentially infected fungal-infected waxworms compared to uninfected (average ratio of 2.8 to 1, respectively). *S. glaseri* IJs did not exhibit preference for one fungal-infected host over another when both were presented simultaneously, and in general invaded fungal-infected hosts in greater numbers than uninfected hosts. The positive response to fungal-infected hosts may be due to short-term increases in carbon dioxide production from fungal-infected waxworms (IJ exposure occurred 24 hours after fungal exposure). These results have interesting relevance to our understanding of competition and niche partitioning in entomopathogens. Future investigations will focus on whether this apparent attraction continues as the fungal infection progresses, and whether these infection decisions are consistent across EPN species with different host ranges and foraging strategies.

Poster – Nematodes Wednesday 16:45 **N-06**

Perspectives of entomoparasitic nematode, *Steinernema feltiae* using to control main pest insects of vineyards in Georgia

Manana Kakhadze, Tsisia Chkhubianishvili, Mariam Chubinisvili, Iatamze Malania, Rusudan Skhirtladze, Iren Rijamadze, Matia Matiasvili and Levan Ninua

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The date on susceptibility of entomoparasitic nematode (EPN) - *Steinernema feltia* to the dangerous pest of vine – the grape berry moth, *Lobesia botrana (Lepidoptera: Tortricidae)* have presented. This insect is the important pest of viticulture regions in Eastern Georgia. The pest damages the grape, especially during the ripping phase when the larvae of third generation appear in mass. No chemical pesticides are recommended for treatment of vineyards. At that time it is advisable to use of biological means such as EPN. Generally *S.feltiae* is considered as the safe biological agent for men and environment to control pests. As the results with microscopic investigations of pathological material the typical situation of invasion, causing by parasitic nematode, has recovered in the grape berry moth body. The invasive ability of new nematode generation, isolated from insect has been established in laboratory. The means on possibility of nematode suspensions insertion in the integrated pest management (IPM) of vineyards from pest organisms have been marked. The EPN pathology was examined toward the important pest insects causing the great damage to vine and others plants - the vine floury mealybug, *Planococus ficus* and the vine pulvinaria mealybug, *Neopulvinaria innumerabilis, (Homoptera: Coccidae).* In paralelly the search and isolation of EPN from the vine growing regions of Eastern Georgia has conducted. The results of preliminary investigations allow to make conclusion that some kind of soils in East Georgia are inhabitation for parasitic nematodes, which may be considered as biological control agents against the pest insects in vineyards.

Poster – Nematodes Wednesday 16:45 **N-7**

Control of diapausing larvae of *Cydia pomonella* in the field using two Chilean strains of entomopathogenic nematodes.

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The entomopathogenic nematodes *Steinernema feltiae* (strain N22) and *Steinernema australe* (strain N3), were tested in the field for efficacy in controlling cocooned larvae of the codling moth *Cydia pomonella* L. Cardboard bands were stapled to the trunks of apple trees as refuges for overwintering larvae. In winter, the bands were sprayed with 100000, 200000, 300000 and 400000 infective juveniles (IJ)/ band. Larvae were removed from cardboard bands 96 hours later and mortality assessed. Regardless of dose, both nematodes caused high mortality on codling moth, with 78-96% and 68-71% mortality for *S. feltiae* and *S. australe*, respectively. Subsequently, 1000000 *S. feltiae* IJ were sprayed onto both cardboard band and surrounding areas of the tree trunk. After 96 hours, cocooned larvae were removed from the cardboard band and bark. Mortality of larvae was higher on the treated *vs* control trees (64 *vs* 9%, respectively) and was higher under the cardboard bands *vs* the bark (79 *vs* 52%, respectively). The results suggest that both *S. feltiae* and *S. australe* are potential biological control agents against overwintering codling moth larvae.

Poster – Nematodes Wednesday 16:45 **N-8**

Selection of native isolates of entomopathogenic nematodes to control the Chilean grape weevil (*Naupactus xanthographus*)

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In Chile, grapevine crop is affected by the Chilean grape weevil ("burrito o mulita"), *Naupactus xanthographus*, pest of primary agricultural and quarantinary importance. Its control is difficult mainly for the underground habits of the larvae, avoiding the conventional insecticides. Entomopathogenic nematodes (EPN) are an alternative as biological control, capable to find their host and move within the soil profile. The Insect Pathology Program CTCB-INIA, currently has a collection of 101 Chilean strains of EPN. Then, the objective of this research was to determine the capacity of parasitism and pathogenicity against larvae of *N. xanthographus* within the Chilean collection. A screening was performed with 101 strains, by placing third or fourth larval stages in containers with moistened pasteurized soil, and then inoculated with a concentration of 100 dauers. The larvae were incubated on chamber at 15 ± 2 °C, and recording daily mortality during 10 d. Those strains showing the highest mortality, the lethal dose (LD_{50} and LD_{90}) was determined by using 0, 5, 10, 20, 40, 80 and 160 dauers/larvae. Sixty six isolates showed some degree of pathogenicity against larvae, 30 of them exceeded 60%, highlighting *Steinernema unicornium* (QU-N85) with 80%, and *S. feltiae* (QU-N21) with 70% mortality. The LD_{50} and $LD_{90}$for QU-N85 and QU-N21 were 28.9 and 21.8 dauers/larvae and 79.8 and 165.0 dauers/larvae, respectively. Therefore, there are EPN able to control the Chilean grape weevil and be an option for pest control.

Poster – Nematodes Wednesday 16:45 **N-9**

Susceptibility of eggs of *Sphenophorus levis* (Coleoptera: Curculionidae) to *Steinernema brazilense* (Rhabditida: Steinernematidae)

Lucas Detogni Simi[1,3], Luis Garrigós Leite[2], Renata Marraschi[2], Fernanda Polastre Pereira[2], Mariana Garcia Martínez-Silva[2], Ana Paula Santos-Bartels[2], Roselaine Nunes da Silva Bueno[2], Antonio Batista Filho[2]

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Sphenophorus levis is an important soil pest of sugarcane in Brazil. The adult lives underground and laid the eggs in the rhizome. This behavior makes the insect a potential target for

use of entomopathogenic nematodes. The aim of this study was evaluating the susceptibility of *S. levis* eggs to *Steinernema brazilense* strain IBCB n6, in sugarcane stalks were artificially infested. The eggs were laid along the 3 days period that stalks were kept exposing to adults in the laboratory rearing, in Instituto Biológico, Campinas, SP. The nematode strain was obtained from the Entomopathogens Collection of the Instituto Biológico, Campinas, SP. In the bioassay, each stalk was artificially infested with 5 eggs, each one set in a 0,5 cm deep role made in the rhizome. The stalks were covered with soil inside pots containing 800 g of sandy soil moistened to 10%. *S. brazilense* suspended in water + Tween 80® 0,1% was sprayed on the soil at concentrations of 3 and 6 IJs/cm^2. The control was sprayed with water + Tween 80® 0,1%. The mortality was assessed 7 days after the nematode application, by opening stalks and evaluating the number of hatched larvae. The control showed 53,3% of hatching larvae, not differing statistically from both concentrations of *S. brazilense*, 3 and 6 IJs/cm^2, with 46,67 and 50% of hatching larvae, respectively. The eggs not hatched showed no evidence of nematode penetration.

Poster – Nematodes Wednesday 16:45 **N-10**

Nematicidal activity of the *Bacillus thuringiensis* to *Meloidogyne incognita* (Nematoda: Meloidogynidae)

Diouneia Lisiane Berlitz[1,2], Cássio de Souza da Silva[1,2], Maximiano Correa Cassal[1], Rita de Cássia Santin[3], Alexandre Guimarães[3], Aida Teresinha Santos Matsumura[3] and Lidia Mariana Fiuza[1]

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The phytonematode *Meloidogyne incognita* infect the roots of crops forming galls, caned be limiting factor in cotton and peanut production, causing great yield losses. The entomopathogenic bacterium *Bacillus thuringiensis* can produce Cry proteins toxic to nematodes. This work evaluated, *in vitro*, news strains of *B. thuringiensis* expressed the *cry* 14 genes, against juveniles of the *M. incognita*. The *Bt* 3434-2P and *Bt* 2974-11P strains were grown in glucosed medium at 28°C, 180rpm and 48 hours, where the mixture of vegetative cells, spores and endotoxins crystals was centrifuged and five concentrations adjusted from 1.10^6 to 1.10^{10} cells/mL. Ten *M. incognita* juveniles (J2) were applied in Elisa plate, in triplicate, and 80µL of bacterial suspension. In the control, the bacterial suspension was replaced with sterile distilled water. The experiment was mantained 28°C, 75% RH and scotophase. The mortality analysis was performed 24 hours after treatment application. The corrected mortality (CM) was calculated using Abbott's formula. The highest concentration showed 91% CM and concentration of 1.10^6 cells/mL, 9% CM by *Bt* 3434-2P. The strain of *Bt* 2974-11P, at 1.10^{10} cells/mL caused 93% CM and 18% CM in the concentration 1.10^6 cells/mL. These results indicate, for the first time, that Cry proteins have the potential to control plant-parasitic nematodes, especifically to *M. incognita.*

POSTER SESSION 2 Wednesday, 16:45 – 18:45

VIRUSES

Poster – Viruses Wednesday 16:45 **V-20** **STU**

Mode of inheritance of resistance to a nucleopolyhedrovirus in the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae)

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The smaller tea tortrix, *Adoxophyes honmai,* is the most important pest of tea cultivation in Japan. *A. honmai* has acquired resistance against chemical pesticides, and biological control is now used to control the pest. To determine whether *A. honmai* can also acquire resistance against baculovirus agents, a field-collected *A. honmai* population was selected with a 70% lethal concentration (LC_{70}) of *A. honmai* nucleopolyhedrovirus (AdhoNPV) in the laboratory. The population developed resistance against AdhoNPV, and the LC_{50} of AdhoNPV-selected strain (resistant strain; R-strain) was significantly higher than that of the nonselected strain (susceptible strain; S-strain) after six generations. The selection was carried out for 15 years, and after 146 generations the R-strain showed over 190,000-fold higher resistance against AdhoNPV than the S-strain. In this study, the mode of inheritance of resistance was examined using offspring of reciprocal F_1 crosses between the R-strain and S-strain and backcrosses between F_1 and S-strain individuals (BC). LC_{50} values of F_1 for both ♂R×♀S and ♂S×♀R showed no significant difference (7.85×10^6 and 4.91×10^6 occlusion bodies/ml, respectively). All LC_{50} values for BC combinations [♂(♂R×♀S)×♀S, ♂S×♀(♂R×♀S), ♂(♂S×♀R)×♀S, ♂S×♀(♂S×♀R)] were also not significantly different from each other and lay between that of the S-strain and F_1. Slopes of dose-mortality regression lines for F_1 were less steep than that of the S-strain. Similarly, all regression lines for BC were also less steep than that for F_1. These data suggest that the inheritance of resistance by *A. honmai* against AdhoNPV is not sex-linked and may be due to a polygenic trait.

Poster – Viruses Wednesday 16:45 **V-21** **STU**

Insect transposons: natural tools potentially involved in the evolution of baculovirus

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Transposons are mobile DNAs spread in most organisms including some viruses. The ability of these sequences in mobilizing can be a decisive factor in evolutionary processes. In Eukarya, transposable elements (TEs) are a significant percentage of genomes showing a great diversity in gene content, size and mechanism of transposition. According to the above, TEs are classified into two main groups: Class I (retrotransposons) and Class II (DNA transposons).

Insect cells are the best system to study and produce baculoviruses. Baculoviridae is a large family of insect viruses that infect and kill different species of Lepidoptera, Hymenoptera and Diptera, containing double-stranded circular DNA genomes of 80,000-180,000 bp with 80 to 180 genes. These variations in gene number suggest that structural mutations are one of the major sources in the emergence of new viral species, and the transposition events potential causes of these effects. In fact, transposons have been detected within baculovirus genomes. Among them, piggybac, TED, TCp3.2 and TC14.7 are TEs described in baculoviruses.

In previous works we isolated from insect cells 3 TEs of Class I and 2 TEs of Class II. In this study we present the complete sequence analyses of them and discuss the potential role of transposons in virus evolution.

Poster – Viruses Wednesday 16:45 **V-22** **STU**

Baculovirus diversification

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From local adaptations to speciation, organisms evolve and diversify at different evolutionary levels. The understanding of the selective pressures leading to species diversification is a complex field, due to the entanglement of numerous factors. By their nature, viruses provide a confined system of interactions to study diversification from micro to macroevolution. Indeed, virus ecological niches are clearly defined by their hosts and consequently these should primarily drive virus evolution. The broadly described insect virus family *Baculoviridae,* and especially the sub-group infecting lepidopterans, provides a large quantity of molecular and ecological data to test hypotheses on ecological speciation. Here, we created two databases. The first one contains sequences of 4 core genes (polh, lef-8, lef-9, pif-2) from ~500 baculovirus isolates. We generated DNA sequences for around 100 isolates and collated all the sequences available in public databases. The second database associates the host ecological data to each isolate, including the family of each host, insect distribution and insect host plant families. With the sequence database, we reconstructed a robust and large baculovirus isolate phylogeny, on which we delimited virus species, clarifying baculovirus taxonomy. Then, we conducted comparative phylogenetic methods to plot the host ecological data onto the phylogeny generated. We found that in general, hosts primarily induced baculovirus species speciation over a short timeframe. But on a larger evolutionary scale, the insect-host co-evolutionary relationship signal is confused and host distribution or insect host plant specificity have also driven the evolutionary history of baculoviruses.

Poster – Viruses Wednesday 16:45 **V-23**

Baculovirus gene Ac109 is required for occluded virus production and budded virus replication

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ORF109 in *Autographa californica* nucleopolyhedrovirus is a core gene that encodes a structural protein essential for viral dissemination into cell culture. Ac109 function is still unknown and contradictory results have been published about its influence in budded and occlusion derived virus production. We constructed a bacmid with a cloramphenicol resistance cassette replacing the gene in order to investigate the consequences of deleting Ac109 in the baculovirus genome. Ac109 knock out bacmid (109KO) produced non-infectious budded viruses and this infectivity could be rescued *in trans* by ac109 gene under the regulation of its own promoter but not when the gene was regulated by OpIE2 promoter or when the deleted gene was complemented with its homologous in SfMNPV. In addition, the Ac109 protein truncated in one of both ends was unable to complement *in cis* the 109KO. On the other hand, we studied the capability of the 109KO virus to produce occlusion bodies. Transmission electron microscopy assays revealed just the presence of empty polyhedra in transfected cells. Besides, we found preoccluded viruses in the nucleus of some cells and plenty of bundles of nucleocapsids without envelopes in others. We further investigated the subcellular localization of the protein alone and in the context of the infection and we determined that Ac109 is a citoplasmatic protein that localizes in the cell's nucleus only when viral factors are present. Taken together, these data show that Ac109 plays an essential role in both viral phenotypes and is involved in the first and last stages of the cell infection.

Poster – Viruses Wednesday 16:45 **V-24**

An *ac34* deletion mutant of *Autographa californica nucleopolyhedrovirus* exhibits delayed late gene expression and a lack of virulence *in vivo*

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Ac34 and its homologs are highly conserved in all sequenced alphabaculoviruses. In this paper, we show that *ac34* transcripts were detected from 6 to 48 h post-infection (p.i.) in *Autographa californica nucleopolyhedrovirus* (AcMNPV)-infected Sf9 cells. Ac34 localized to both the cytoplasm and the nuclei of infected cells but was not a viral structural protein. To determine the function of *ac34* in the viral life cycle, an *ac34*-knockout AcMNPV (vAc34KO) was constructed. Compared with wild-type and repair viruses, vAc34KO exhibited an approximately 100-fold reduction in infectious virus production. Further investigations showed that the *ac34* deletion did not affect the replication of viral DNA, polyhedron formation or nucleocapsid assembly but delayed the expression of late genes, such as *vp39*, *38k* and *p6.9.* Bioassays revealed that vAc34KO was unable to establish a fatal infection in *Trichoplusia ni* larvae via *per os* inoculation. Few infectious progeny viruses were detected in the hemolymph of the infected larvae, indicating that the replication of vAc34KO was attenuated. These results suggest that Ac34 is an activator protein that promotes late gene expression and is essential for the pathogenicity of AcMNPV.

Poster – Viruses Wednesday 16:45 **V-25 STU**

Complementation of p74 KO AcMNPV using a transgenic cell line

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Baculoviruses are large dsDNA viruses that have been isolated from Lepidoptera, Diptera, and Hymenoptera. These viruses are widely used as protein expression systems, platform to develop vaccines and gene therapy, and as bioinsecticides to their natural hosts that causes significant losses in agriculture. There are two phenotypes present in the viral cycle. The budded viruses (BVs) are involved in the systemic spread while Occlusion Derived Viruses (ODVs) cause the primary host-specific infection. ODVs are covered by a protein coat that protects them against environmental conditions, forming the Occlusion Bodies (OBs). The OBs are ingested by larva and are dissolved in the insect gut, releasing the ODVs which then specifically bind and fuse to the brush border microvilli of columnar cells in the midgut epithelium. This different tropism between the two phenotypes is controlled by the proteins present in their viral envelopes. PIFs proteins (Per Os Infectivity Factors) are present in ODVs coat, which are essential for the baculovirus entry. To date there are six PIFs reported: PIF0 or P74, PIF1, PIF2, PIF3, PIF4 and PIF5 or ODV-E56. *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) is used in America to control a soybean plague. With the aim to develop new bioinsecticides with improved qualities, we generated a transgenic Hi5 cell line that expresses P74 protein of AgMNPV. These cells were used to multiply AcMNPV knock out in its *p74* gene. The validation of transgenic cells and the biological effects of trans-complementation were tested.

Poster – Viruses Wednesday 16:45 **V-26**

Functional studies on the *per os* infectivity factor 3 (PIF3) of HearNPV

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PIF3 is one of the six conserved *per os* infectivity factors (PIFs) of baculovirus. In this study, PIF3 of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) was analyzed by infectivity bioassays of a series of recombinant viruses harboring various PIF3 truncation/substitution mutants. The results demonstrated that the N-terminal region (L26-Y45) and C-terminal region (T160-Q199) are essential for HearNPV oral infectivity. In the C-terminal T160-Q199 region, there are three conserved cysteines (C162, C164 and C185). Our results showed that substitutions of C162 or C164, predicted to be involved in disulfide bond formation, led to severe decrease in HearNPV *per os* infectivity. Mutation of C185, predicted not to be involved in disulfide bond formation, did not affect the *per os* infectivity. The data suggest that disulfide bonds are important for PIF3 conformation and function. Immunofluorescence assays showed that all the mutants did not affect the subcellular localization of PIF3 to the nuclear ring zone region of the infected cells. Western blot results showed that except C162G and C185G, all other mutants failed to incorporate PIF3 into ODVs, which resulted in the impaired oral infectivity of the latter. The data provide insights for future study of PIF3 function.

Poster – Viruses Wednesday 16:45 **V-27**

***Autographa californica* multicapsid nucleopolyhedrovirus late genes mediate Arp2/3 complex nuclear relocation during virus infection**

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One of the unique features of *Autographa californica* Multicapsid Nucleopolyhedrovirus (*Ac*MNPV) infection is the nuclear relocation of G-actin and Arp2/3 complex, which subsequently induces actin polymerization in host nucleus and assists *de novo* synthesized nucleocapsid assembly. Ohkawa *et al* previously identified six *Ac*MNPV early genes mediate G-actin nuclear relocation, however the mechanism for Arp2/3 complex nuclear relocation remains unknown. In order to track Arp2/3 complex subcellular distribution changes during *Ac*MNPV infection, we cloned *arpc1* (*p41*) subunit of Arp2/3 complex from Sf9 cells and utilized P41 to represent Arp2/3 complex. Unlike G-actin nuclear relocation which is mediated by viral early genes, P41 appears to relocate to host nucleus by viral late genes, as cell fractionation assay indicates aphidicolin (a DNA replication inhibitor that blocks baculovirus late genes expression) significantly reduces the nuclear fraction of P41 in *Ac*MNPV infected cells, in comparison with DMSO-treated infected cells. In accordance with cell fractionation assay, immunofluorescence microscopy further reveals P41 relocates to peri-nucleus region at 12-24 hours post infection, whereas aphidicolin blocks the nuclear relocation and P41 maintains a cytoplasmic distribution pattern, which is similar to uninfected cells. This research will continue to identify which viral late genes are involved in Arp2/3 complex nuclear relocation, and the significance of this research will provide new insight into how baculovirus harnesses host genes to serve for its replication.

P

oster – Viruses Wednesday 16:45 **V-28**

Analysis of induction and suppression of apoptosis in the *Lymantira dispar* Ld652Y cells infected with nucleo-polyhedroviruses

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Ld652Y cells derived from the gypsy moth, *Lymantria dispar*, readily undergo apoptosis following infection with a variety of nucleopolyhedroviruses (NPVs), while homologous *L. dispar* multicapsid NPV (LdMNPV) infection of Ld652Y cells results in the production of a high titer of progeny viruses without induction of apoptosis. In this study, we found that two inhibitor of apoptosis proteins encoded by LdMNPV, Ld-IAP2 and Ld-IAP3, were incapable of suppressing apoptosis of Ld652Y cells in transient expression assay. We identified a novel LdMNPV apoptosis suppressor gene, *apsup*, which suppressed apoptosis of Ld652Y cells induced by infection with vAc□p35, the *Autographa californica* MNPV defective in antiapoptotic gene *p35*. Apsup also suppressed apoptosis of Ld652Y cells induced by nonviral stimuli such as exposure to actinomycin D and UV. RNAi-mediated *apsup* ablation induced apoptosis of LdMNPV-infected Ld652Y cells. Although database search revealed that Apsup homologues were encoded in several baculoviruses and one poxvirus, no functional analyses have been performed for any of these Apsup homologues. Domain search of Apsup identified no characteristic domains that were linked to possible functions. To investigate the apoptosis suppression mechanism of Apsup, we cloned *L. dispar* initiator caspase (*ld-dronc*) and effector caspase (*ld-caspase-1*) which served as the dominant factors in apoptosis signal pathway. We analyzed the interactions between Apsup, Ld-Dronc, and Ld-Caspase-1 to provide insight into virus antiapoptotic functions against apoptosis-dependent host defenses.

Poster – Viruses Wednesday 16:45 **V-29**

Host insect liquefaction in infections with *Condylorrhiza vestigialis multiple nucleopolyhedrovirus*: effect of possible variation in cathepsin (*v-cath*) and chitinase (*chiA*) genes

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The lepidopteran *Condylorrhiza vestigialis*, commonly known as the Alamo Moth, is a major defoliating pest of Poplar crops in Brazil. The use of biopesticides for the control of this and other pests has advantages over chemicals, principally because they are of narrow spectrum. The baculovirus *Condylorrhiza vestigialis multiple nucleopolyhedrovirus* (CoveMNPV), pathogenic to the *C. vestigialis* caterpillar, has been applied in Poplar plantations in Southern Brazil to combat this pest. Due to the occurrence of virus-killed larvae presenting symptoms of liquefaction and non-*liquefaction* of the integument, the present study aims to investigate the presence or absence in the CoveMNPV genome of cathepsin (*v-cath*) and chitinase (*chiA*) genes, responsible for post-mortem liquefaction of the integument of the caterpillar, from both field samples and *plaque purified virus clones*. Initially, comparative analyses were performed using restriction profiles of DNA samples extracted from viral occlusion bodies, cleaved with five restriction endonucleases: *Bam*HI, *Bst*EII, *Eco*RI, *Hin*dIII and *Pst*I. Some profiles possessed submolar bands, indicating the presence of genotypic variation within the respective isolate. We therefore conducted *in vitro* purification of viral clones. One of the clones was utilized as a template for PCR, using primers *targeting* the CoveMNPV *gp64 gene.* The PCR product was then used as a probe in a Southern blot containing *Pst*I-cleaved DNA from the viral clones and field samples. Hybridizing bands will be cloned and the inserts sequenced. The sequence will then be analyzed for the *lef-7*/*gp64* intergenic region and the presence or absence of the *v-cath* and *chiA* genes in the CoveMNPV genome.

Poster – Viruses Wednesday 16:45 **V-30**

Study of polyhedrin functional complementation among nucleopolyhedroviruses

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Baculoviruses produce occlusion bodies (OB) of a characteristic size and shape, Specific interactions between the occlusion matrix protein and other viral components that control virion occlusion and OB morphology are still not well understood. Polyhedrin is a conserved protein among nucleopolyhedroviruses (NPVs) and shares above 80% identity. Although this high level of similarity may suggest a nonspecific mechanism of interaction between virion and occlusion protein, primary amino acid sequence affects occlusion body morphology and it has been hypothesized that polyhedral growth is initiated by specific interactions between polyhedrin molecules and the virion envelope. In order to evaluate the interactions between virions and heterologous polyhedrin, a stably transformed *T. ni* cell line containing the polyhedrin gene (*polh*) of AcMNPV (*Autographa californica Nucleopolyhedrovirus*) was obtained. In these cells the *polh* expression was dependent upon transactivation of a modified AcMNPV *polh* promoter containing a homologous repeat (AcMNPV *hr1*). Clonal cell lines were infected with AcMNPV(*occ*$^-$) and selected for their capacity to produce OBs. Selected clones were checked by PCR and western blot. The clonal lines presenting high levels of polyhedrin expression were then infected with *Anticarsia gemmatalis Nucleopolyhedrovirus* (AgMNPV) (*occ*$^-$). Regular size OBs were apparent, indicating that AcMNPV polyhedrin can occlude AgMNPV virions. These hybrid OBs killed *Anticarsia gemmatalis* larvae when administered *per os*. Since High Five™ cells can support replication of a wide range of baculovirus species, the obtained cell line represents also a valuable resource to occlude other *polh* defective virions.

Poster – Viruses Wednesday 16:45 **V-31**

Development of a cell line derived from High Five™ for simple titration of baculovirus

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Lepidopteran cell line Tn5B1-4 (commercially known as the High Five) is highly susceptible to several baculoviruses and usually provides superior production of recombinant proteins when compared with other insect cell lines. For this reason, High Five cells are primarily used for protein expression. Baculovirus stock titers are usually determined in Sf-9 or Sf-21 cells lines, since these cells have a more regular morphology and the plaques produced by AcMNPV (*Autographa californica* nucleopolyhedrovirus) are easily distinguished. Baculovirus uptake by the High Five cells is much more efficient than in Sf-9. In consequence, titers determined in High Five cells, expressed as plaque forming units, are usually higher and, probably, reflect the numbers of infectious virions more closely. In order to simplify the titration procedure of baculovirus in High Five cells, a transformed cell line expressing the enhanced green fluorescent protein (eGFP) under the control of the very late polyhedrin (*polh*) promoter of AcMNPV was developed. The obtained cell line was infected with an AcMNPV expressing the red fluorescent protein (dsRed). The GFP expression pattern showed correlation with the infection reporter (dsRed), demonstrating the homogeneity of the cell line. In order to begin to evaluate the possibility of titrating other baculovirus species, the obtained cell line was infected with *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV). The green fluorescent plaques were readily visible, indicating that AgMNPV factors can transactivate the AcMNPV *polh* promoter. These results demonstrate that this transgenic cell line can be used to simplify titration and the susceptibility of the parental line to other baculovirus species might extend its application.

Poster – Viruses Wednesday 16:45 **V-32**

Enhanced production of Porcine circovirus type 2 capsid protein by the fusion expression with baculovirus partial polyhedrin

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Porcine circovirus type 2 (PCV2) is a non-enveloped circular single-stranded DNA virus associated with postweaning multisystemic wasting syndrome, which is considered to be an important infectious swine viral disease. PCV2 capsid protein encoded by ORF2 is a major structural protein and expected as the high immunogenicity protein. For the efficient production of capsid protein, the recombinant baculoviruses were generated to express ORF2 of various fusion forms with a partial polyhedrin of *Autographa californica* nucleopolyhedrovirus. Recombinant capsid protein was produced successfully with fusion form of partial polyhedrin and the yield was high like as shown visible clearly on SDS-PAGE. Production and status of recombinant fusion proteins in insect cells were confirmed by SDS-PAGE and Western blot analysis using His-tag antibody, anti-ORF2 monoclonal antibody and anti-PCV2 serum. Optimal multiplicity of infection (MOI) and infection time for the production of recombinant capsid protein were determined as 5 MOI and 4 days, respectively. The results suggest that the fusion expression with polyhedrin is able to increase the production of recombinant PCV2 capsid protein in insect cells.

Poster – Viruses Wednesday 16:45 **V-33**

Comparison of expression of haemagglutinin from H5N1 influenza virus by three different baculovirus expression systems.

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Insect baculoviruses are popular expression vectors for diagnostic reagents and vaccines. For example the human papilloma virus vaccine Cervarix is based on baculovirus expression of HPV capsid protein. We expressed the haemagglutinin (HA) from a highly pathogenic avian influenza (HPAI) H5N1 virus isolate, all from the polyhedrin promoter, in three different formats to compare their relative efficacy. These three were HA overexpression using the standard expression system, expression of HA as a GP64 display in the form of HA fused to GP64 (HA:GP64) and expression as a VP39 capsid protein display in which HA is fused to the amino end (HA:VP39) or carboxy end of VP39 (VP39:HA). To facilitate detection of the various products the HA was tagged with FLAG. For HA overexpression using anti FLAG antibody both the full length glycosylated and non-glycosylated forms were detected (as confirmed by tunicamycin treatment) in addition to a smaller fragment suggestive of cleavage at the HA1/HA2 cleavage site. For the display viruses, bands suggestive of HA:GP64 were detected by both anti FLAG and anti GP64 antibodies while VP39:HA and VP39:HA bands were detected by both anti FLAG and anti VP39 antibodies. All suggestive HA bands were also

detected by anti HA polyclonal and monoclonal antibodies. The HA from the HA overexpression and HA:GP64 fusion had biological activity as demonstrated by haemadsorption of chicken red blood cells to infected cells and by haemagglutination. Based on Western immunoblotting the GP64 display virus (HA:GP64) provided the highest amount of product, followed closely by HA overexpression.

Poster – Viruses Wednesday 16:45 **V-34**

Development of an immunological technique for detecting granulovirus infection in *Tuta absoluta* larvae (Lepidoptera: Gelechiidae)

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Phthorimaea operculella granulovirus (PhopGV) has been used for controlling larvae of different moths from Gelechiidae's family as *Tecia solanivora* and *P. operculella* in several countries of South America. This virus can also be pathogenic for *Tuta absoluta* larvae, one of the most important pests of tomato crop. However, viral isolates from this insect has not been reported nowadays, possibly due to the small larvae size and the difficulty for detecting the virus. In this sense, the aim of the present work was to develop an economic, fast and accurate immunological technique for granulovirus detection in *T. absoluta* larvae. First step was the production of polyclonal antibodies against one PhopGV isolate by using hens and rabbits. Production system in hen was selected as the most efficient and simple method for antibodies production and purification. Then a Dot blot test was developed and antibodies affinity and specificity were tested. No cross-reactivity was detected with proteins of healthy *T. absoluta* larvae, while a high affinity for viral granulovirus particles was observed, by recognizing different granulovirus isolates. A minor degree of reactivity was observed with a *S. frugiperda* nucleopolyhedrovirus. These results demonstrate the ability of antibodies to recognize viral proteins, specially the granulin. The performance of the Dot Blot was satisfactory in terms of sensitivity, detecting at least $5x10^4$ Occlusion Bodies/mL (650ng/mL) of PhopGV in a purified virus suspension. Developed inmunological test represents an inexpensive, accurate and rapid alternative for routine detection of granulovirus in *T. absoluta* larvae.

Poster – Viruses Wednesday 16:45 **V-35**

Replication of two entomopoxviruses in CF70 cells derived from the eastern spruce budworm, *Choristoneura fumiferana*

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The subfamily of *Entomopxvirinae* encompasses viruses in three genera based on the order of the host insect. Viruses in the genus *Betaentomopoxvirus* infect lepidopteran and coleopteran insects. We tested the CF70 cell line for permissiveness to two entomopoxviruses (EPVs). The cell line was maintained in HyClone SFX serum free medium (pH 6.1-6.4) and was originally derived from ovaries of the spruce budworm. It was permissive to at least two entomopoxviruses from *Choristoneura fumiferana* (CFEV) and from the red hairy caterpillar, *Amsacta moorei* (AMEV). CFEV replicates productively with production of spheroids in most cells and by day 3-4 post infection, the virus causes the cells to form giant syncytia. While this phenomenon has been reported for orthopoxviruses, it has not been seen before in EPVs. For example, Ectromelia induces cell-cell fusion under neutral pH but needs a high concentration of virus particles at the cell surface. We have not observed syncitium formation in CF70 cells infected with AMEV. However, while the latter virus replicates productively in the cell line, it also induces apoptosis at late stages of infection. We found out that the virus not only induces apoptosis, it appears to depend on a component(s) of this pathway to produce high titres of progeny virus. Several RNA viruses appear to induce apoptosis in infected cells without affecting production of progeny particles. This phenomenon has also been observed with some other DNA viruses but not with poxviruses in general.

Poster – Viruses Wednesday 16:45 **V-36**

Biochemical characterization of the 3C-like protease from *Ectropis obliqua* virus

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Viruses of the order *Picornavirales* encode large polyproteins, and they all require polyprotein proteolytic processing by viral 3C or 3C-like (3CL) proteases. In this study, we have identified and characterized the 3CL protease of *Ectropis obliqua* virus (EoV) of the newly established family *Iflaviridae* (order *Picornavirales*). We have determined EoV 3CL to mediate both the *cis*- and *trans*-cleavages when expresses in *E. coli* and also have identified the cleavage sites of EoV 3CL by the N-terminal sequencing and mutational analyses. Additionally, we demonstrated that both the *cis*- and *trans*-cleavages occur at the same positions. Mutagenesis data provided evidence to suggest the three conserved residues (H2261, D2299 and C2383) are critical for the protease activity. Furthermore, we have characterized EoV 3CL's biochemical properties and responses to various protease inhibitors. This study is the first time to identify a 3CL cysteine protease in the family *Iflaviridae* and elaborate this 3CL protease's characteristics, which should advance our understanding not only to EoV but also to other iflaviruses.

Poster – Viruses Wednesday 16:45 **V-37**

Concomitant natural infections with the mermithid *Strelkovimermis spiculatus* and a mosquito iridescent virus in *Culex pipiens*

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Culex pipiens is a vector desease and important pest in their distribution area in the world. In Argentina, among the most common breeding sites of this mosquito specie is the man-drainage ditches located in suburban areas of the cities from which a high number of adults mosquitoes emerge throughout the year. During a survey for natural parasites and pathogens of *Cx. pipiens* in La Plata city, Argentina, we collected larvae of *Cx. pipiens* with mixed infections with a mosquito iridescens virus (MIV) and the neotropical mermithid specie *Strelkovimermis spiculatus* in suburban ditches with permanent water, rich in organic matter during 2010 and 2011. Prevalence data of virus and nematode in mosquito population was registered in each sample. This mermithid was observed in 87.5 % of samples positive for this virus. It was confirmed that this virus belongs to the family Iridoviridae by electron microscopy and molecular methods. Forty five larvae with patent infection of MIV were dissected under stereomicroscope, 42% of wich were recorded with the presence of *S. spiculatus*. Transmission tests were conducted at laboratory to study the interaction virus-mermithid in the mosquito host. Preliminary transmission tests suggested that preparasitic stages (J2) of *S. spiculatus* could be one way of entry through the cuticle for the MIV particles to hemocoele.

Poster – Viruses Wednesday 16:45 **V-38**

A new insect rhabdovirus from *Culex tritaeniorhynchus* mosquitoes utilizes host's nuclear splicing machinery

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Among members of the order *Mononegavirales*, RNA splicing events have been found only in the family *Bornaviridae*. Here, we report that a new rhabdovirus isolated from the mosquito *Culex tritaeniorhynchus* replicates in the nuclei of infected cells and requires RNA splicing for viral mRNA maturation. The virus, designated *Culex tritaeniorhynchus* rhabdovirus (CTRV), shares a similar genome organization with other rhabdoviruses, except for the presence of a putative intron in the coding region for the L protein. Molecular phylogenetic studies indicated that CTRV belongs to the family *Rhabdoviridae*, but it is yet to be assigned a genus. Electron microscopic analysis revealed that the CTRV virion is extremely elongated, unlike virions of rhabdoviruses, which are generally bullet shaped. Northern hybridization confirmed that a large transcript (approximately 6,500 nucleotides [nt]) from the CTRV L gene was present in the infected cells. Strand-specific reverse transcription-PCR (RT-PCR) analyses identified the intron-exon boundaries and the 76-nt intron sequence, which contains the typical motif for eukaryotic spliceosomal intron-splice donor/acceptor sites (GU-AG), a predicted branch point, and a polypyrimidine tract. *In situ* hybridization exhibited that viral RNAs are primarily localized in the nucleus of infected cells, indicating that CTRV replicates in the nucleus and is allowed to utilize the host's nuclear splicing machinery. This is the first report of RNA splicing among the members of the family *Rhabdoviridae*.

Poster – Viruses Wednesday 16:45 **V-39**

Nucleotide sequence variations of the major structural proteins (VP15, VP19, VP26 and VP28) of white spot syndrome virus (WSSV), a pathogen of cultured *Litopenaeus vannamei* in Mexico

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White spot syndrome virus (WSSV) was first reported in farmed *Litopenaeus vannamei* stocks in Sinaloa and Sonora, Mexico during 1999 and continues to cause severe shrimp losses. WSSV genes encoding nucleocapsid (VP26 and VP15) and envelope proteins (VP19 and VP28) of a Mexican isolate were cloned in the pMosBlue vector. The nucleotide sequences of these genes were compared with WSSV isolates in GenBank. VP15 is highly conserved, and VP26 showed 99% homology to a Chinese isolate. The VP28 fragment demonstrated 100% homology to the majority of the isolates analysed (UniProt accession no. Q91CB7), differing from two Indian WSSV and one Chinese WSSV isolates by two non-conserved and one conserved replacements, respectively. Because of their highly conserved nature, these three structural proteins are good candidates for the development of antibody-based WSSV diagnostic tools or for the production of recombinant protein vaccines to stimulate the quasi-immune response of shrimp. In contrast, VP19 of the Mexican isolate was distinguishable from almost all isolates tested, including an American strain of WSSV (US98/South Carolina, GenBank accession no. AAP14086). Although homology was found with isolates from Taiwan (GenBank accession no. AAL89341) and India (GenBank accession no. AAW67477), VP19 may have application as a genetic marker.

THURSDAY 9th

Workshop II Thursday, 08:00 - 10:00
DBI Division

OIE-notifiable aquatic invertebrate diseases: a Latin American perspective

Workshop II Thursday, 08:00 **142**

Listed diseases and the global trading of aquatic crustaceans

Stentiford, G.D.

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Expansion of the global shrimp farming industry since the early 1980s has aligned with an increasing perception of the potential for trans-boundary movement of disease agents in broodstock, larvae and commodity products. To take account of this, pathogens, and the diseases that they cause in susceptible hosts, have been listed in international legislative frameworks such as that provided by the World Health Organisation for Animal Health (OIE) and the European Union (e.g. EC Directive 2006/88). Diseases are listed by the OIE in accordance to defined criteria associated with their potential for negative consequence, spread, and diagnosis. The OIE list is updated in the Manual of Diagnostic Tests for Aquatic Animals, published at approximately 3 year intervals. Occurrence of a listed disease within one of the 178 current Member Countries requires official reporting via the Competent Authority of that country, to the OIE, followed by regular updating until the disease is eradicated or otherwise. Similar arrangements also occur where regional listing of diseases occurs (e.g. within the EU) whereby Member States report to the European Commission *and* (where appropriate), to the OIE. Reporting aims to limit the extent of the outbreak and further spread of the pathogen via natural routes and trading of animals. It further provides a global surveillance system for known and emerging pathogens of concern to farmed and wild aquatic animals. However, the presence of a listed disease within a country can also lead to trading restrictions associated with live animals and their products. Currently, 6 viral diseases (WSD, TS, YHD, IHHN, IMN, WTD), one bacterial disease (NHP) and one fungal disease (crayfish plague) are listed by the OIE. Despite this system, the rapid global spread of emergent pathogens continues to occur. The emergence of Infectious Myonecrosis (IMN) caused by infectious myonecrosis virus (IMNV) in Brazil in 2003, and its subsequent spread to *P. vannamei* farms operating in Indonesia by 2006 epitomises the 'lesson not learnt' from previous experiences with illegal trading of infected broodstock. Estimated losses due to IMN in Indonesia already amount to over $1bn, posing a significant threat to food security from the shrimp sector. Here, I will discuss the implications of OIE disease listing for the trading of crustaceans and their products in the global industry and overview the interaction between net exporting nations in Latin America, and net importing nations in the EU with regard the regional legislation outlined in EC Directive 2006/88.

Workshop II Thursday, 08:30 **143**

Presence of OIE –Notifiable viral pathogens in crustaceans from Argentina

Sergio Martorelli

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Since 2003 two of the most important commercially caught shrimp in Argentina (the Argentine stiletto shrimp, *Artemesia longinaris,* and the Pink shrimp *Pleoticus muelleri)* together with another crustaceans of ecological interest were examined for

parasites, epibionts and pathogens. In 2008 several specimens of the penaeid shrimp *A. longinaris* from the Bahia Blanca estuary were found with numerous black spot in the cephalothorax shell.
In some of these specimens histological sections stained whit H&E showed the presence of inclusion bodies type Crowdy A, coincident normally with infections of the White Spot Syndrome Virus (WSSV). In a first molecular study, using a commercial WSSV kit, two specimens were found positives for this virus. To confirm and double check the presence of this virus in *A longinaris* and in other two crustaceans, that were found also with spots in their cuticles (the grapsoid crab *Cyrtograpsus angulatus*, and the introduced shrimp *Palaemon macrodactylus*), several specimens of each of these three species were analized in collaboration with Dr. Overstreet in the Gulf Coast Research Laboratory (USA). In this study three PCR methods and qPCR were used. Of all the samples 56% of *A longinaris*, 67% of *C. angulatus* and 40% of *P macrodactylus* were positive for WSSV. in addition 30% of *A longinais* was also positive for IHHNV. Most of the WSSV positive crustacean has low copy numbers of the virus and some explanations for this finding are presented. Finally characteristics of this outbreak in wild crustacean are discussed with new data, and three possible routes of introduction of the virus in Argentina were analyzed.

Workshop II Thursday, 08:30 **144**

First survey of notifiable viral diseases of crustaceans in wild red shrimp *Pleoticus muelleri* in the San Jorge Gulf, Argentina.

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A first survey was conducted to determine the health status of wild Patagonian red shrimp, populations in the San Jorge Gulf, for white spot syndrome virus (WSSV), infectious hypodermal and haematopoietic necrosis virus (IHHNV), infectious mionecrosis virus (IMNV), Taura syndrome virus (TSV) and yellow head virus (YHV). The red shrimp *Pleoticus muelleri* (Bate, 1888) is abundant in Argentinean waters and represents one of the most important fisheries in the Argentine Sea. Shrimps are mostly exported in frozen condition. World trade in frozen shrimp products has lead to concern regarding the risk of spread of shrimp viruses worldwide, by the international movement of these commodities.

As the San Jorge Gulf zone's disease situation was unknown, Argentina's Under secretariat of Fisheries and Aquaculture in association with the animal health authorities (National Animal Health Services – SENASA) and the colaboration of INIDEP (National Institute for Fisheries Research and Development), designed and conducted an initial field survey to study wild populations of Patagonian red shrimp for evidence of viral diseases of crustaceans.

One hundred and seventy (170) shrimps were sampled considering 2% prevalence and 95 % confidence level (OIE 2009). Samples of *Pleoticus muelleri* adults and sub-adults were collected from 5 locations throughout the San Jorge Gulf. Samples of gill tissue and pleopods from three individuals were taken and pooled. Testing for all viruses involved the use of NAD (nucleic acid detection) PCR, RT-PCR techniques. If any sample was found positive each individual of the pool was retested and confirmed by sequencing. A histopathological examination was performed as well on all the samples. All laboratory tests determined the non-existence of typical clinical signs or internal histopathological presentation compatible with the viral diseases studied, and neither the specific sequences of the responsible viral pathogens were detected at any site of the fishing hauls. Although these primary results confirmed the absence of clinical disease further active surveillance actions with annual sampling is being undertaken to be more vigilant on the risk of introduction and spread of crustaceans' viruses that may represent a potential threat to wild populations of this economic important Argentinean commodity shrimp.

Workshop II Thursday, 09:00 **145**

Epidemiology, histopathology and ultrastructure of *Bonamia exitiosa* infected *Ostrea puelchana* and *Bonamia* sp infected *O. stentina* from San Matías Gulf, Patagonia, Argentina

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Oysters are one of the best known groups of molluscs and one the highest importance in commercial aquaculture. However, flat oysters have almost disappeared due to high mortalities produced by parasites of the genus *Bonamia* that severely affected oyster farms around the world. In Argentina, experimental marine aquaculture began with the native specie, *Ostrea puelchana*, in 1995. One year later an abnormal mortality was reported in San Antonio Bay (San Matías Gulf) which aetiological agent was *B. exitiosa*. The presence of this pathogen was the first record for this genus in the SW Atlantic Ocean and it is the only OIE Notifiable Disease of aquatic organisms known in Argentina. The culture area inside San Antonio Bay was identified as the focus of infection from which the parasite spread. Infection radiated from this focus through the oysters beds located at the SW and NE of the bay. Another specie of oyster without commercial value, *Ostrea stentina* cohabits with *O. puelchana* in shallow waters of the San Matías Gulf. The density of the populations of this species in some beds of the gulf has dramatically decreased in the last decade. Recent histopathological studies revealed the presence of a *Bonamia*-like pathogen similar to that affects *O. puelchana.*In this presentation we review epidemiological information concerning the local specie of *Bonamia* obtained until 2011, and also we described the histopathology of *O. puelchana* infected by *B. exitiosa* and the ultrastructural description of this pathogen.

Symposium X Thursday, 08:00 - 10:00
Microbial Control Division
Microbial Control – The Latin American Way

Symposium X Thursday, 08:00 **146**

Latin American successes in microbial control – a view from outside

Trevor Jackson

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The tropical and subtropical climates in Latin America have favored a great diversity of life, including a wide variety of entomopathogenic microbes. Led by enthusiastic pioneers like Flavio Moscardi and Sergio Batista Alves, Latin Americans have adopted microbial control as an important component in integrated pest management programs. Fungi are now widely used in many countries, especially *Beauveria bassiana* for insect and mite control in protected cultivation and crops and *Metarhizium anisopliae* as a microbial pesticide against sugar cane and pasture spittlebugs. The nucleopolyhedrovirus of the velvetbean caterpillar,*Anticarsia gemmatalis* (AgMNPV) has been used extensively for protection of soybeans in Brazil, Argentina, Paraguay, Colombia and Mexico. Bacteria (mainly *Bacillus thuringiensis*) have been used in many crops and also widely used against blackflies and mosquitos for public health. The successful use of microbial control in Latin America is not widely known outside the continent as it is based on local

innovations and practical methods for implementation. It has also involved close partnerships between the scientists, producers and farmer groups leading to numerous bio-factories throughout the continent.

Symposium X Thursday, 08:30 **147**

The use of *Bacillus thuringiensis* based biopesticide for small-scale growers in Brazil.

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Spodoptera frugiperda (Smith) is responsible for significant losses in maize production (up to 52%). Its control is mainly achieved using chemical insecticides. Small-scale producers of maize in the state of Ceará, Northeast of Brazil, suffer great losses because of this insect pest. A small biofactory ($25m^2$) was built to produce *Bacillus thuringiensis* (Bt) based biopesticide, using rice as a substrate. Bt strain 344 was grown in rice enriched with carbon (maize glucose), nitrogen (yeast), and mineral salts (all expressed in $g.L^{-1}$ - 0.002 g of $FeSO_4$, 0.02 g of $ZnSO_4$, 0.02 g of $MnSO_4$, 0.3 g of $MgSO_4$ (pH adjusted to 7.2). Rice was mixed with the medium and sterilized at 121°C for 30 min. Fifty grams of rice were used in each plastic bag mixed with the medium. After Bt inoculation on rice, sealed plastic bags were incubated at 30°C for 4 days. During 5 months about 4.700 doses (one dose=1 ha) were produced. During the Bt production period, seminars and short courses were taught in all Agricultural Extension Units of Ceará (Ematerce). The region of Crato(a city in the state) received the largest amount of Bt doses (1.800) and Tauá and Iguatú (two other cities), the smallest amount, 200 doses each. The plastic bags containing Bt biopesticide were frozen till the use in the field. To reach all regions of Ceará, freezers were placed in each strategic point till distribution to the farmers. A new formulation (powder) is being developed.

Symposium X Thursday, 09:00 **148**

Progress and opportunities in microbial control in the Chilean fruit industry

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The fruit sector in Chile is a very dynamic activity, reaching 1.5% of national GDP and 32% of the agricultural and forestry sector's GDP. The production is about 4,300,000 tons of fruits and growing at 4.5% annual rate since 1990. About 80% of the country production is exported, both as fresh and processed fruit. Thus, Chile is the main fruit exporter from the South hemisphere, ranking first world exporter for grapes and plums, the second world supplier of avocados, the third of kiwi fruit and blueberry, the fourth of cherry, and the sixth in apple. To keep this industry open to the world, the sanity and pest management is a major concern for the country. Biological control of fruit pests has been used for almost 110 years in Chile; however the main focus was conventional control against above ground pests. The first reference of entomopathogens (EP) is back to 1957, but the study and research of EP for fruit pest control is rather recently, since 1996. Main pests under control or development by EP are quarantine insects, either native or introduced such as Apple moth (*Cydia*), Melybugs (*Pseudococcus*), Grape weevil (*Naupactus*), Raspberry weevil (*Aegorhinus*), Fuller's rose weevil (*Asynonychus*), Black vine weevil (*Otiorhynchus*), Ghost moth (*Dalaca*), White grubs (*Hylamorpha and Phytoloema*) and Yellow jacket (*Vespula*). The use of EP fungi and nematodes have been used with promissory results, therefore some of these isolates are currently used commercially but largely remain as a potential tools for most of the Chilean fruit industry.

Symposium X Thursday, 09:30 **149**

Microbial Control of Insects: A Brazilian Perspective

Daniel Ricardo Sosa-Gómez[1], Marcos Rodrigues de Faria[2], Bráulio Santos[3], José Eduardo Marcondes de Almeida[4] and Luís Garrigós Leite[4]

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In Brazil, the most important systems in recent years for applied microbial control of insects have been sugar cane, pastures, soybean, poplar reforested areas and rubber tree plantations. Fungi are the microbial agents most widely used countrywide. Annually, *Metarhizium* is applied on approximately 700,000 ha, mainly in the States of São Paulo, Pernambuco, Alagoas, Sergipe, Mato Grosso do Sul, Mato Grosso, Goiás and Minas Gerais, and most often used in sugar cane fields to control cercopid leafhoppers. The two major reasons for reduced use of the AgMNPV baculovirus in recent years are constant infestations since 2002 of soybean looper (*Pseudoplusia includens*), and the expectation for Bt-soybean introduction in the Brazilian market. As an example of baculovirus use, one company sold AgMNPV for use on 225 thousand ha in the 2009/10 soybean season, on 187 thousand ha in 2010/11, and 192 thousand ha in 2011/12. In the last five years, a baculovirus from *Condylorrhiza vestigialis* (Lepidoptera: Crambidae), a poplar tree pest, has also been applied on 300 $ha.year^{-1}$ in Paraná and Santa Catarina states (União da Vitoria, São Mateus do Sul, Porto União and Canoinhas). In rubber tree plantations, the fungus often identified as "Sporothrix insectorum" is applied in 3,000 $ha.year^{-1}$ to control the *Leptopharsa heveae* (Tingidae), with most of its production at the Instituto Biológico, São Paulo. The treatment of these biological agents under Brazilian law as genetic patrimony resources imposes serious restrictions on their production and commercialization and prevents their possible sale or use outside of Brazil.

SIP Annual Business Meeting Thursday, 10:30 **150**

The three Gs: Personal reminiscences of invertebrate cell culture pioneers: Goldschmidt, Gao, and Grace

Karl Maramorosch

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It was my good fortune to meet and know personally the three invertebrate cell culture pioneers , Goldschmidt, Gao, and Grace. Richard Goldschmidt was a prominent geneticist in Germany in 1914. After a visit to Japan, he was prevented from returning home by the outbreak of World War I. He remained for 4 years in the United States, working at the Osborn Laboratory of Yale University. There he came under the influence of Ross G. Harrison, who in 1904 pioneered animal cell culture. At Yale Goldschmidt successfully maintained silkworm sperm in culture, but in his publications he never mentioned Dr. Rhoda Erdmann, who, I suspect, actually carried out the experiments. In 1956 Gao in Wuhan, China, published his successful cultivation of silkworm cells in Acta Virologica, in English, but his work was completely ignored outside China. When I met Gao in 1982 in Wuhan, he told me that he was trained in USA, worked at Rockefeller Institute in Princeton and at the Osborn Laboratory at Yale University before returning to China. He was inspired by William Trager and Ross G. Harrison, becoming China's leading invertebrate cell culture pioneer. In 1956 T. D. C. Grace, from CSIRO, Canberra, Australia, joined my laboratory at Rockefeller University for 2 years. He devised a cell culture medium in which he maintained a beating moth heart for one year. After returning to Australia, through constant transfers to fresh culture media, and, in his words, "benign neglect", he obtained continuous moth cell cultures. The subsequent expansion of invertebrate cell culture involved a large number of women scientists, including the Nobel

laureate Rita Levi Montalcini, who in 1969 published the first of a dozen papers on in vitro studies of the embryonic nervous system of *Periplaneta americana*, that led to her milestone discovery of the nerve growth factors. Goldschmidt and Erdmann never returned to insect cell culture, but Erdmann became Europe's most prominent human cell culture researcher. She organized the first, second and third European tissue culture congresses and created the Cell Culture section of Cell Research at Berlin's Cancer Institute.

Workshop III Thursday, 14:00 - 16:00
DBI and Bacteria Divisions

Use of RNAi to control insects or diseases of insects

Workshop III Thursday, 14:00 **151**

Why is it untrue that killing the messenger doesn't solve the problem

Esteban Hopp

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Among gene regulation mechanisms, RNA interference (RNAi) is a process that specifically down regulates gene expression by preventing the messenger RNA (mRNA) to translate into a protein. This means that by killing the messenger, it is possible to get rid of a given problem that is solved by knocking down gene expression. Historically, it was also known by co-suppression, post transcriptional gene silencing and quelling. Knock down is carried out by two types of small RNA – microRNA (miRNA) which are mainly involved in development and cell gene expression regulation and small interfering RNA (siRNA) mainly involved in defense -by binding to the specific mRNA. RNA interference has an important role in defending cells against parasitic genes – viruses and transposons – but also in directing development as well as gene expression in general. The RNAi activation is induced by long double-stranded RNA (dsRNA) molecules which are processed by the enzyme Dicer, which cleaves them into ~20 nucleotide size single-stranded siRNAs and incorporated into the RNA-induced silencing complex (RISC). As consequence, post-transcriptional gene silencing occurs when this siRNA anneals with a complementary mRNA sequence and induces cleavage by Argonaute, the RNA-degrading subunit of the RISC complex. In some conditions this process can be spread systemically. This rather gene specific action of RNAi makes it a valuable biotechnological tool, because synthetic dsRNA can be used to induce knock down of specific genes of interest.

Workshop III Thursday, 14:15 **152**

RNAi products platform for invertebrates' health and targeted pest control

Eyal Ben-Chanoch

Beeologics, Inc., USA

Ribonucleic Acid interference (RNAi) applications for invertebrates are on the scientific agenda since RNAi was initially introduced. The work done on c-elegance not only was awarded with the Nobel Prize in 2006, but also triggered many initiatives that focused on how the RNAi mechanism can be used for pest management. RNAi based products are been developed by Beeologics to control honeybee viruses and parasites. The first and most advanced product to be introduced is Remebee® – an anti-viral agent fed to the bees protecting them from acute infection caused by the Israeli Acute Paralysis Virus (IAPV). This virus has associated with honeybee mortality and identified as one of the potential participating factors of Colony Collapse Disorder (CCD). The technology platform built in the creation of Remebee covers many of the aspects of new product introduction and now supporting the R&D of several complementary RNAi products; for the honeybees as well as a variety of application for targeted pest control.

Workshop III Thursday, 14:45 **153**

Design and evaluation of a strategy for the control of cotton weevil, based on dsRNAs ingestion that induces gene silencing

Ricardo Salvador[1, 2], Natalia Almasia[2], José Niz[1], Marcelo Berretta[1], Cecilia Vazquez-Rovere[2], Alicia Sciocco-Cap[1] and Esteban Hopp[2]

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A. grandis is widely distributed in the Americas and represents an important pest on cotton production. Control strategies based on chemical methods are difficult due to the emergence of resistance and larvae endophytic lifestyle. Considering the reduced availability of *A. grandis* genetic sequences, a transcriptome analysis was performed. Sequences obtained from midgut revealed a wide variety of putative proteins involved in digestion, defense and detoxification, *Bt* toxin binding and RNA interference processes. Selected midgut genes were amplified and used to synthesize dsRNA *in vitro* and by plant expression. Bioassays in *A. grandis* larvae and adults were conducted in order to evaluate the efficacy of dsRNA administrated by oral route. Preliminary results indicate that dsRNA is a valuable tool to design new strategies of cotton weevil control.

Workshop III Thursday, 15:00 **154**

The mode of action of dsRNA for control of western corn rootworm (*Diabrotica virgifera virgifera*) larvae

Gerrit Segers, Parthasarathy Ramaseshadri, Ron Flannagan, William Moar and Renata Bolognesi

Monsanto Company, 700 Chesterfield Pkwy W, Chesterfield, MO, USA, 63017

Double-stranded RNA (dsRNA) is known to be effective in Diabrotica larvae via oral delivery. After ingestion, the dsRNA is thought to be taken up by the insect midgut cell and processed by the native RNAi machinery, which leads to specific knockdown of the target mRNA. The knockdown effect can cause lethality if the target mRNA encodes a protein with an essential function in the insect. Although processing of the dsRNA and target mRNA degradation appear to be conserved in western corn rootworm, some of the key steps in this process, such as uptake of dsRNA into midgut cells, are still unknown. In this presentation, we will present data on the dsRNA uptake mechanism and its selectivity for molecules of different sizes, as well as, the detailed morphological and cellular defects that lead to western corn rootworm (WCRW, *Diabrotica virgifera virgifera*) death upon ingestion of dsRNA targeting an essential gene.

Workshop III Thursday, 15:30 **155**

Pyramiding dsRNA with Bt to control corn rootworm

William Moar, Tom Clark, Graham Head, Gerrit Segers, Renata Bolognesi and Ron Flannagan

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The expression of dsRNA in crop plants, such as maize, to control damaging insect pests such as western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) represents a new class of insect control traits with a high degree of insect specificity. As with any new plant-expressed insect control trait, the potential for WCR to evolve resistance to dsRNA needs to be considered. This presentation will discuss information regarding pyramiding WCR-active dsRNA with Bt Cry proteins for increased efficacy and decreasing the potential for resistance development while maintaining a relative high degree of specificity in host spectrum.

Contributed Papers Thursday, 14:00-15:30

Microbial Control 3

Contributed Microbial Control 3 Thursday, 14:00 **156**

First comparative transcriptomic analysis of wild adult male and female *Lutzomyia longipalpis*, vector of Visceral Leishmaniasis

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Leishmaniasis is one of the most diverse and complex of all vector-borne diseases worldwide. It is caused by parasites of the genus *Leishmania*, obligate intramacrophage protists characterised by diversity and complexity. Its most severe form is visceral leishmaniasis (VL), a systemic disease that is fatal if left untreated. In Latin America VL is caused by *Leishmania infantum chagasi* and transmitted by *Lutzomyia longipalpis*. This phlebotomine sandfly is only found in the New World, from Mexico to Argentina. The disease is sustained through diverse complex interactions between the *Leishmania* parasites, the vector and the mammalian host. The availability of high-throughput approaches has aided the identification of pertinent vector molecules that affect the development of the *Leishmania* parasite, its transmission and its establishment in the mammalian host. In this study, unbiased high-throughput pyrosequencing technology was used to accomplish the first comparative transcriptomic analysis of wild male and female adult *Lu. longipalpis* from endemic (Posadas, Misiones) and non-endemic (Lapinha Cave, Minas Gerais) VL locations in Argentina and Brazil, respectively. Sequences associated with metabolic, cellular, localisation and biological regulation processes were identified, among others. Interestingly, these included sequences homologous to detoxification enzymes (eg., cytochrome P450) and cellular stress proteins (eg., heat shock proteins). Nevertheless, most of the identified sequences corresponded to proteins with unknown function. Since this is the first transcriptomic analysis that includes male and female *Lu. longipalpis*, genes that were expressed in common represent possible candidates for vector control, among others.

Contributed Microbial Control 3 Thursday, 14:15 **157**

Build up of pathogens within outbreak populations of native insect populations in modified land in New Zealand

Sean D.G. Marshall[1], Richard J. Townsend[1], Andreas Leclerque [2], Regina G. Kleespies[2], Jessica E. Dunbar[3], Tracey L. Nelson[1] and Trevor A. Jackson[1]

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The Gondwanaland remnant of Aotearoa-New Zealand developed a unique flora and fauna over >60 million years. When settlers introduced European grasses and clovers they initially grew spectacularly in the absence of their usual pest complex but some native insects were capable of adapting to the new resources and often destroyed the developing pastures until pesticides and IPM measures were introduced. Recently, extensive land modification through 'flipping' of swamps to create new pastures has led to unexpected outbreaks of pests on the South Island West Coast. After initial vigorous pasture growth, two species of manuka beetle, *Pyronota festiva* and *P. setosa* (Coleoptera: Scarabaeidae) reached unprecedented levels (exceeding >1300/m² within 5 years of land development), resulting in extensive damage (up to 60% of pastures) and production losses (up to 30%) in untreated areas. This appears to be a case of "enemy release" as the insects thrived in a pathogen and predator free environment produced by the land change. In the first years after modification, no pathogens were found in the invading populations. Gradually pathogens including *Beauveria brongniartii*, '*Rickettsiella pyronotae*' and a microsporidian disease have been isolated and are spreading through the pest populations. We will present and discuss our findings in relation to the appearance of microbial pathogens and their role in regulating new pest populations.

Contributed Microbial Control 3 Thursday, 14:30 **158**

Expression of *Bacillus thuringiensis* toxin Cry1Ia7 in *Pseudomonas fluorescens* confers protection against UV radiation

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The *Bacillus thuringiensis* Cry1Ia7 insecticidal protein is toxic against several lepidopteran species, including the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). Cry1Ia7 does not form part of the paraesporal crystal but is expressed and secreted during the vegetative phase. This, along with its rapid degradation upon exposure to solar ultraviolet radiation hinders its use as a biological insecticide. To address this problem, *cry1Ia7* was cloned into a UV-resistant *P. fluorescens* strain. Cry1Ia7 was expressed and encapsulated in vegetative *P. fluorescens* cells following established protocols. The toxicity of Cry1Ia7 protein produced in *P. fluorescens* was purified in an amylose affinity column using a maltose binding protein (*Pf*-MBP-Cry1Ia7) and compared with that of Cry1Ia7 expressed and encapsulated in *P. fluorescens* (*Pf*-Cry1Ia7). Both proteins were active against *L. botrana*, and their mean lethal concentrations (LC_{50} values) were not statistically different (10.63 and 12.6μg/ml, respectively). The activity of free *Pf*-MBP-Cry1Ia7 protein was reduced 10-fold following exposure to a UV source; however, the LC_{50} of *Pf*-Cry1Ia7 encapsulated in *P. fluorescens* was not significantly affected by exposure to UV, due to the photoprotection offered by *P. fluorescens* encapsulation. This system of protein production can be used as a model for other *Bacillus thuringiensis* proteins that would benefit from improved protection against solar UV radiation.

Contributed Microbial Control 3 Thursday, 14:45 **159**

A Novel formulation of biopesticide

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Mosquitoes are known to be vectors of a number of viral and protozoa related diseases such as cerebral inflammation, yellow fever, and malaria. Usually chemical products are being used to control of mosquitoes. These chemicals have high costs, no long-lasting effects and they cause human and environmental problems. Therefore, biological control of mosquitoes is important, and the objective of this study is to develop formulation of an effervescent granule of lyophilized biopesticide against to mosquitoes that does not have harmful effects on environment and human health. At the previous study it was obtained three new strains of *Bacillus sphaericus* (MBI5, 6, 7). MBI5, MBI6 and MBI7 strains were reported as biopecticide against to mosquitoes. These strains were also determined as a new sub-type of *Bacillus sphaericus* in the literatures. A new commercial formulation was developed from the selected bacterial strains (MBI5, MBI6 and MBI7). The present study is to formulate a lyophilized effervescent (MBI5, MBI6 and MBI7) granule as a delivery system which provides

rapid dissolving of biopesticides. Lyophilization process is a special technique that enables to maintain stability of sensitive biopesticides. This novel formulation is provided in the form of a single use dosage as granules. The advantages of lyophilized biopesticide, first of all an economical biopesticide with high applicability is obtained, which does not have harmful effect on environment and human health, and secondly easily dissolve and can lead to effective pest management to control of mosquitoes.

Contributed Microbial Control 3 Thursday, 15:00 **160**

Use of microbial insecticides for the control of filarial vector, *Culex quinquefasciatus*

Kadarkarai Murugan

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Mosquito-borne diseases are major component of communicable diseases in India and in other parts of the world. Insecticides from microbial origin are eco-friendly method of control of insects that provides alternatives to synthetic insecticides. In the present study to test the effect of microbial insecticides such as *Bacillus thuringiensis* israelensis (Bti), *Bacillus sphaericus* (Bs)*, Spinosad* (Spd) and *Beauveria bassiana* (Bb) against the *Culex quinquefasciatus* at the laboratory and in the field. Microbial insecticides at different concentrations showed considerable larval mortality. Lethal concentrations (LC_{50} and LC_{90}) were worked out and LC_{50} and LC_{50} values against 4^{th} instars after the treatment of Bti were 2.512, 2.217; Bs were 8.37, 21.57; Spd were 0.34, 0.12 and Bb were 7.09, 14.37, respectively. Field trial was also conducted at the endemic area and to study the efficacy against larval population. Population reduction of larvae was noted after 24h, 48h and 72h treatment. Higher efficacy was found with Bti were 82, 92, 97%; with Spd were 76, 93, and 97%, 65, 79, 82, and Bb 61, 82, 88%, respectively. Combined treatment had higher efficacy (95.5%), (100%), (100%) than the individual treatments during 24h, 48h and 72h treatment. Hence, these microbial larvicide's will help to kill the spectrum of mosquito larvae in the breeding habitats and also the active ingredients in the microbial insecticides which affect the biochemical and physiological processes of insect system.

Contributed Papers Thursday, 14:00-15:30

Viruses 5 *Functional Genomics II*

Contributed Viruses 5 Thursday, 14:00 **161**

Analysis of IE0 and IE1 transactivation of *Autographa californica* multiple nucleopolyhedrovirus early promoters

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The alphabaculovirus AcMNPV produces essential transregulatory proteins called IE0 and IE1. Both IE0 and IE1 have been shown to transcriptionally transactivate viral early promoters but to date no promoters have been shown to be specific to either IE0 or IE1 transactivation. However, levels of expression from genes transactivated by IE0 and IE1 varied significantly. Past studies expressed IE0 and IE1 under control of the native *ie0* or *ie1* promoters and it was unknown if differences in promoter transactivation was due to different functional properties or simply due to varying levels of IE0 and IE1. To address this issue *ie0*, *ie0*$^{M\text{-}A}$ or *ie1* were placed under the control of identical promoters, either *ie1* or *gp64,* to obtain comparable levels of expression in transient transactivation analyses. The *ie1* promoter expresses at higher levels than the *gp64* promoter. 19 early gene target promoters were chosen based on a microarray analysis of the whole AcMNPV genome. The CAT gene was placed under control of each early promoter and was analyzed for differences in transactivation by IE0, IE0$^{M\text{-}A}$, and IE1. Few differences were observed between IE0, IE0$^{M\text{-}A}$, and IE1 when expressed at relatively high levels (*ie1* promoter). However, when expressed at low levels (*gp64* promoter), some promoters such as *lef6* and *p35* were more highly transactivated by IE0 and IE0$^{M\text{-}A}$. These results suggest that at low levels of expression, similar to what is observed in the first few hours of viral infection there are differences in the level of activation of specific genes by IE0 or IE1.

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Stability regulation of baculovirus-encoded N-WASP homologous protein P78/83

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The actin polymerization induced by baculovirus nucleocapsid protein P78/83 during virus infection shows specific temporal and spatial patterns. At early phase of virus infection, transient actin polymerization occurs at host cytoplasm and pushes viral nucleocapsid towards nuclear membrane; at late phase of virus infection, secondary actin polymerization occurs at host nucleus and assists the assembly of *de novo* synthesized nucleocapsid. However, the mechanism of the dynamic actin polymerization during baculovirus infection remains unknown. According to our preliminary data, host cell rapidly degrades P78/83 through its N-terminal sequence, whereas viral nucleocapsid protein BV/ODV-C42 stabilizes P78/83 through its interaction with P78/83. This phenotype suggests the stability and functional status of P78/83 are interactively regulated by host cell and virus, which may possibly be attributed to the specific temporal and spatial patterns of actin polymerization during baculovirus infection. This research will continue to investigate the detailed mechanism of how P78/83 is regulated by host cell and virus, as well as its impact upon actin polymerization and virus replication. The significance of this research will help understand the formation of dynamic actin polymerization during baculovirus infection, and reveal a novel regulation method of actin polymerization through protein degradation at cell biology level.

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Deletion of orf114 of AcMNPV diminishes its per os infectivity by reducing the numbers of ODVs in occlusion bodies

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Autographa californica multiple nucleopolyhedrovirus (AcMNPV) *orf114* is one of the highly conserved unique genes in lepidopteran group I nucleopolyhedrovirus (NPV), whose roles remain unknown. To study the function of *ac114* in the virus life cycle, an *ac114* knockout bacmid was generated. Fluorescence and light microscopy showed that *ac114* deletion mutant was able to produce infectious budded viruses (BVs) and occlusion bodies (OBs). Titration assays demonstrated that *ac114*-deletion virus had the similar growth kinetics to the control virus during the phase of infection. Electron microcopy observation indicated that *ac114* did not affect the morphogenesis of BVs and occlusion derived viruses (ODVs), while the numbers of ODVs per OB of the *ac114*-deletion virus were significantly lower than those of the control virus. Bioassay showed that *ac114* deletion did not change the killing speed of AcMNPV in *Spodoptera exigua* larvae, but reduced its viral infectivity significantly. These data indicated that *ac114* is an auxiliary gene which facilitates embedding of ODVs into the OBs and thus affect its infectivity.

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A Group II alphabaculovirus core gene, MacoNPV-A *pif-5* (*odv-e56*), cannot repair the essential *per os* infectivity function of an AcMNPV-*pif5* knockout virus in *Trichoplusia ni* larvae.

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Recently the baculovirus core gene, *odv-e56,* was demonstrated to be essential for oral infectivity of AcMNPV and was designated as *per os infectivity factor-5* (*pif-5*). In a series of ongoing studies of baculovirus gene function we have used an *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) Bacmid system to generate gene knock-out and repair viruses repaired with either the respective AcMNPV (Group I) gene or its *Mamestra configurata* nucleopolyhedrovirus–A (MacoNPV-A) homolog. Among these constructs we developed a polyhedrin+, GFP tagged AcMNPV-Bacmid virus in which *pif5* (*ac148,odv-e56*) was knocked out ($vAc^{pif5null}$) as well as repair viruses with wild type AcMNPV *pif5* ($vAc^{pif5null\text{-}Acpif5HA}$) and *macoApif5* ($vAc^{pif5null\text{-}macoApif5HA}$). In this study, we have confirmed the essential requirement of AcMNPV PIF5 for oral infectivity of AcMNPV ODV. Fluorescence and electron microscopy as well as $TCID_{50}$ and qPCR assays revealed that the knock-out virus, $vAc^{pif5null}$, and repair viruses showed normal levels of budded virus replication and produced normal occlusion bodies in cell culture. However, occlusion bodies of the *pif5* knock-out virus were unable infect *T. ni* larvae when inoculated *per os* and there was no evidence that ODV entered midgut epithelial cells. The repair virus $vAc^{pif5null\text{-}Acpif5HA}$ however demonstrated wild type oral infectivity. Although *pif-5* has been demonstrated not to be an integral part of the PIF complex in ODV, the Group II homologue, MACOAPIF5, was unable to restore oral infectivity of the $vAc^{pif5null\text{-}macoApif5HA}$ in *T. ni* larvae.

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Characterization of novel components of the baculovirus *per os* infectivity factor (PIF) complex

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Baculovirus occlusion-derived virus (ODV) infects insect midgut cells under alkaline conditions, a process mediated by highly conserved *per os* infectivity factors (PIFs). Previously, a multi-molecular complex composed of PIF1, PIF2, PIF3, and P74 (PIF0) was identified, which was proposed to play an essential role during ODV entry. Recently, more proteins have been identified that play important roles in oral infectivity. Identification of all components of the PIF complex is crucial In order to understand the ODV entry mechanism. Co-immunoprecipitation (CoIP) combined with proteomic analysis was used to identify the components of the *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) PIF complex. PIF4 and P95 (AC83) were identified as novel components of the PIF complex, and this was confirmed with Blue-native PAGE and a second CoIP. PIF5 on the other hand was not present in the complex. Deletion of the *pif4* gene impaired complex formation, but deletion of *pif5* did not. Differentially denaturing SDS-PAGE further revealed that PIF4 forms a stable complex with PIF1, PIF2, and PIF3. P95 as well as P74 are more loosely associated with this complex. Three other proteins, AC5, AC68 (recently identified as PIF6), and AC108 (homologue of the recently recognized PIF protein SF58), were also found to be associated with the PIF complex in the proteomic analysis. Finally the functional significance of the PIF protein interactions is discussed.

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HSP70 Induction during baculovirus infection

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Baculoviruses are arthropod-specific double-stranded DNA viruses that have been employed as bio-insecticides against crop pests and to produce heterologous proteins in baculovirus expression systems. Although a consensus has emerged on the dominant molecular events driving baculovirus replication *in vitro*, little is known regarding the host-virus interplay in the infected insect. In our previous work, RNA-seq and 2d gel analysis revealed that the inducible 70kDa heat shock protein (hsp70) was among those cellular proteins most up-regulated following productive baculovirus infection. Transcription of *hsp70* is triggered during cellular stress in an hsf-1-dependent fashion, a stress-related transcription factor we have also detected as induced following baculovirus infection. Due to its role in promoting protein sorting and proper folding, we hypothesized that baculoviruses may benefit from the presence of hsp70 during late times of infection, owing to overwhelming translation of viral proteins and their subsequent trafficking through the endoplasmic reticulum. In the present study, insects that were either permissive or non-permissive for infection revealed that induction of *hsp70* was dependent on viral replication. *In vivo* experiments analyzing virus infection demonstrated a progressive induction of *hsp70* over time, culminating in the greatest expression in the fat body prior to the death of the host, a somewhat surprising finding given that the midgut is source of primary infection. These studies add to a growing body of evidence suggesting a method by which baculoviruses may have evolved to subvert host cellular processes to promote efficient viral propagation.

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Identification and characterization of the initiator caspase SfDronc in *Spodoptera frugiperda* and its role in apoptosis induced by *Autographa californica* M nucleopolyhedrovirus

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Initiator caspases are the first caspases that are activated following an apoptotic stimulus. Once activated, initiator caspases cleave and activate downstream effector caspases, which directly cause apoptosis. In *Drosophila*, the initiator caspase Dronc is required for most, if not all, apoptotic cell death. Orthologs of Dronc have been identified in several other insect species, including *Bombyx mori* (BmDronc). We have cloned a cDNA encoding a Dronc homolog from *Spodoptera frugiperda* (SfDronc). The predicted SfDronc protein is 447 amino acids in length and shares 53.6% and 24.8% identity with BmDronc and *Drosophila* Dronc, respectively. Recombinant His-tagged SfDronc cleaved synthetic initiator caspase substrates, but had little activity against effector caspase substrates. Silencing of *Sfdronc* expression by RNAi in Sf9 cells blocked induction of apoptosis by a mutant of *Autographa californica* M nucleopolyhedrovirus (AcMNPV) lacking the anti-apoptotic gene *p35*, or by treatment with UV or actinomycin D. Silencing of *Sfdronc* also reduced cleavage of the effector caspase

SfCaspase-1 in UV-treated cells. Purified recombinant P35 protein from AcMNPV, which is an effector caspase inhibitor, was not able to inhibit the cleavage activity of SfDronc. These results suggest that SfDronc is the major initiator caspase responsible for induction of caspase-dependent apoptosis in *Spodoptera frugiperda*. As such, SfDronc is a likely candidate for Sf-caspase-X, an initiator caspase-like activity responsible for cleavage and activation of Sf-caspase-1 which was first observed by Friesen and colleagues in *Spodoptera* cells more than a decade ago.

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Characterization of the interaction between the AcMNPV sulfhydryl oxidase Ac92 and the *Spodoptera frugiperda* P53 protein

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Orthologs of the *Autographa californica* M nucleopolyhedrovirus (AcMNPV) open reading frame 92 (*ac92*) are conserved in all sequenced baculovirus genomes. The predicted Ac92 gene product is related to flavin adenine dinucleotide-linked sulfhydryl oxidases, which catalyze the formation of disulfide bonds in cysteine-containing peptides. We have shown previously that the active site cysteines in Ac92 are necessary for sulfhydryl oxidase activity and virion morphogenesis, implying that this enzymatic activity is necessary for proper virion assembly. Ac92 is an apparent structural protein found associated with budded and occlusion-derived virions. Ac92 was shown previously to interact with human tumor suppressor protein P53 and enhance human P53-mediated apoptosis in insect cells. We were interested in determining whether Ac92 also interacted with *Spodoptera frugiperda* P53 (SfP53) and whether this interaction affected virus replication. Co-immunoprecipitation experiments involving transiently-expressed proteins verified an interaction between Ac92 and SfP53. This interaction was not disrupted when the cysteines in the predicted active site of Ac92 were altered. However, a mutation in the predicted DNA binding residues of SfP53 eliminated this interaction. Ac92 expressed from its native promoter also interacted with endogenous SfP53 in virus-infected cells, suggesting that SfP53 may be a substrate of Ac92. Silencing the expression of *Sfp53* by RNA interference did not rescue budded virus formation from an *ac92*-knockout virus, and lack of Ac92 did not affect the increased accumulation of SfP53 observed in infected cells. The biological relevance of the physical association of Ac92 and SfP53, including whether SfP53 is a substrate of Ac92, remains under investigation.

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Gut microbiota promotes baculovirus pathogenesis

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Organisms' response to pathogens is reflected in their transcriptional profiles. We designed an expression microarray to decipher the transcriptional response of *S. exigua* to pathogenic baculovirus infections. Agilent 44K *S. exigua* custom microarrays were used to determine the genes differentially expressed in *S. exigua* Se301 cells challenged with a species-specific (SeMNPV), and a non-specific (AcMNPV) baculovirus. In contrast to the expected host transcriptional shut-down, Se301 cells showed relatively balanced numbers of up-regulated and down-regulated genes during the first 36 hours of the infection. Se301 cells responded differentially to SeMNPV and AcMNPV, with more genes being regulated after the challenge with the native virus, though the last observation may be the result of differential timing in infection development. Among the up-regulated unigenes, we found a few *repat* family genes, as well as many genes responsible for signal transduction, ATP processing, chitin structure and metabolism and hormones. Among the down-regulated genes we found many with homology to known immune related genes, like peptidoglycan recognition proteins, lectins, lysozyme-like proteins, scavenger receptors, phenol oxidase pathway enzymes, G-protein receptors as well as antimicrobial peptides, such as attacin, cecropin and lebocin, and genes coding for proteins involved in the melanisation cascade. Given the clear changes in the expression profiles of antimicrobial genes as well as other immune related genes we have further investigated the virus pathogenic process in the absence and presence of intestinal microbiota. We will discuss the importance of intestinal bacteria in viral infections and propose possible mechanisms of viral regulation of the host immune response.

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