47TH ANNUAL MEETING

SIP

MAINZ GERMANY 2014

47th ANNUAL MEETING

of the

SOCIETY FOR INVERTEBRATE PATHOLOGY

and

INTERNATIONAL CONGRESS ON INVERTEBRATE PATHOLOGY AND MICROBIAL CONTROL

Berichte aus dem Julius Kühn-Institut

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Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Sunday – 3 August

9:00-17:30	SIP Council Meeting	P 203
10:00-19:00	Registration	P1
18:00-21:00	Mixer	Alte Mensa

Monday – 4 August

7:30-18:00	Registration	P1
8:30-10:00	**Opening Ceremony** Johannes Jehle, Organizing Committee Jørgen Eilenberg, President SIP Welcome Addresses Student Travel Award Presentation by M. van Oers **Founder's Lecture** James Becnel, Chair of Founders' Lecture Committee Honoree: Alois M. Huger Lecturer: Trevor A. Jackson	P1
10:00-10:30	Break	
10:30-12:30	***Plenary Symposium*** **Microbial Control - from Bench to Business** Potentials for utilizing and controlling insect pathogens *Richou Han* Story of an African firm: 10 years in the biopesticide business – lessons learned along the way *Sean Moore* A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods *Willem J. Ravensberg* BASF Functional Crop Care. Unlocking Agricultural Potential in Soil, Seed and Crop *Sebastian Bachem*	P1
12:30-14:00	Lunch	Mensa
14:00-16:00	***Symposium 1 (Nematode Division)*** **Above and Belowground Interaction, Root-Shoot Interaction, Chemical Signaling** Small molecule signals in nematodes - common motifs and species specific modifications *Stephan H. von Reuss* Olfactory Plasticity in Entomopathogenic Nematodes *Elissa Hallem* Multiple Consequences of Belowground Herbivore Induced Volatile Signals *Jared G. Ali* Root Zone Chemical Ecology; New Techniques for Below Ground Sampling and Analyses of Volatile Semiochemicals *Hans T. Alborn*	P4
14:00-16:00	**Contributed Papers** Bacteria 1 Viruses 1 Fungi 1	 P5 P1 P2
16:00-16:30	Break	
16:30-18:30	***Symposium 2 (Microsporidia Div.)*** **Microsporidiology: Advances in Europe** A new intracellular parasite is a missing link between fungi and microsporidia *Karen L. Haag* Parasite takes fly - A Drosophila model of Microsporidia infection *Sebastian Niehus* White Sea metchnikovellids: morphology, life cycles; potential ancestral features of microsporidia *Yuliya Y. Sokolova* Microsporidia: Pathogens of Opportunity *James J. Becnel*	P3
16:30-18:30	**Contributed Papers** Nematodes 1 Viruses 2 Fungi 2	 P4 P1 P2
20:00-21:30	**Division Business Meetings and Workshops** Microbial Control Diseases of Beneficial Invertebrates Nematodes	 P3 P5 P4

Tuesday - 5 August

7:30-13:00	Registration	P1
8:00-10:00	***Symposium 3 (Fungi Division)*** **Fatal Attraction: Fungi and Odours in deadly Combinations for Pest Control** Conifer - bark beetle - fungus interactions *Tao Zhao* Carbon dioxide as an orientation cue for western corn rootworm and wireworm larvae - implications for an attract and kill approach using entomopathogenic fungi *Stefan Vidal* Different behavioral responses in specialist and generalist natural enemy interactions (predators and fungi) in a strawberry-mite pest system *Stine Kramer Jacobsen* How Fusarium graminearum influences insect-plant interactions *Drakulic Jassy* Plant-microorganism interactions that shape host-plant selection in the grapevine moth *Marco Tasin* Effect of host plant on aphid susceptibility to the fungal pathogen Pandora neoaphidis *Cezary Tkaczuk*	P2
8:00-10:00	**Contributed Papers** Nematodes 2 Viruses 3 Bacteria 2	 P4 P1 P5
10:00-10:30	Break	
10:30-12:30	***Symposium 4 (Virus Division)*** **Small non-coding RNAs as Regulators of Insect Host-Virus Interactions and Immunity** Role of cellular and virus-encoded microRNAs in insect host-virus interactions *Sassan Asgari* Sensing viral RNA in Drosophila melanogaster *Carine Meignin* Small RNA-directed antiviral immunity in disease-vector mosquitoes *Kevin M. Myles* Controlling viral infection in insects *Raul Andino*	P1
10:30-12:30	**Contributed Papers** Microbial Control 1 Diseases of Beneficial Invertebrates 1 Fungi 3	 P3 P4 P2
12:40	Departure of buses for optional excursion + 5K	Univ.
15:00	Departure of buses for BBQ + 5K	Univ.

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AND MICROBIAL CONTROL

PROGRAM and ABSTRACTS

3-7 August 2014

University of Mainz, Germany

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2014

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.

Attendants shall **not take pictures** from projections during the presentations

STU indicates papers being judged for graduate student presentation awards

129 indicates abstract number for ORAL presentation

B-11 indicates abstract number for POSTER presentation

SUNDAY - 3 August

9:00–17:30	SIP Council Meeting	**P203**
10:00–19:00	Registration	**P1**
18:00–21:00	Mixer	**Alte Mensa**

MONDAY - 4 August

07:30-18:00 Registration **P1**

Monday, 8:30-10:00. **P1**

Opening Ceremony and SIP Founders' Memorial Lecture

Opening Ceremonies

Johannes Jehle, Chair, Organizing Committee
Jørgen Eilenberg, President, SIP

Welcome Addresses

Student Travel Award Presentation by M.van Oers

Founders' Memorial Lecture
James Becnel, Chair, Founders' Lecture Committee
Honoree: **ALOIS M. HUGER**
Lecturer: **TREVOR A. JACKSON**

10:00–10:30 **BREAK**

Plenary Symposium Monday, 10:30–12:30. **P1**

Microbial Control - from Bench to Business

Organizer/Moderator: Ralf-Udo Ehlers

10:30 **1 Potentials for utilizing and controlling insect pathogens** Richou Han, Xuehong Qiu and Xun Yan, Guangdong Entomological Institute, 105 Xingang Road West, Guangzhou 510260, China

11:00 **2 Story of an African firm: 10 years in the biopesticide business – lessons learned along the way** Sean Moore, Citrus Research International, Port Elizabeth, South Africa; Rhodes University, Grahamstown, South Africa

11:30 **3 A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods** Willem J. Ravensberg, Koppert Biological Systems, Berkel en Rodenrijs, the Netherlands

12:00 **4 BASF Functional Crop Care. Unlocking Agricultural Potential in Soil, Seed and Crop** Sebastian Bachem, BASF – Limburgerhof, Germany

12:30–14:00 **LUNCH** Mensa

Symposium 1 (Nematodes) Monday, 14:00-16:00. **P4**

Above and Belowground Interaction, Root-Shoot Interaction, Chemical Signaling

Organizers/Moderators: R. Campos-Herrera, F. Kaplan and S. Hazir

14:00 **5 Small molecule signals in nematodes - common motifs and species specific modifications** Stephan H. von Reuss, Max Planck Institute for Chemical Ecology, Department of Bioorganic Chemistry, Jena, Germany

14:30 **6 Olfactory Plasticity in Entomopathogenic Nematodes** Joon Ha Lee and Elissa Hallem, University of California, Los Angeles, USA

15:00 **7 Multiple Consequences of Belowground Herbivore Induced Volatile Signals** Jared G. Ali[1,2], Raquel Campos-Herrera[2,3], Hans T. Alborn[4], Larry W. Duncan[2], Lukasz L. Stelinski[2]; [1]Department of Entomology, Michigan State University, USA; [2]Entomology and Nematology Department, Citrus Research and Education Center, University of Florida, U.S.A.; [3]Departamento de Contaminación Ambiental, Instituto de Ciencias Agrarias, CSIC, Madrid, Spain; [4] Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL, U.S.A.

15:30 **8 Root Zone Chemical Ecology; New Techniques for Below Ground Sampling and Analyses of Volatile Semiochemicals** Hans T. Alborn[1]; Fatma Kaplan[2]; [1]USDA ARS Center for Medical, Agricultural and Veterinary Entomology, Gaines-ville FL, U.S.A.; [2]Kaplan Schiller Research LLC and Biology Dept. University of Florida, Gainesville, FL, U.S.A.

Contributed Papers Monday, 14:00-16:00. **P5**

BACTERIA 1

Moderators: Raffi Aroian and Brian A. Federici

14:00 **9 Discovery of Insecticidal Proteins from Non-Bacillus Bacterial Species** Nasser Yalpani[1]; Dan Altier[1], Jennifer Barry[1], Jarred Oral[2], Ute Schellenberger[2], Adane Negatu[1], Scott Diehn[1], Virginia Crane[1], Gary Sandahl[1], Joe Zhao[1], Dave Cerf[2], Claudia Perez Ortega[3], Mark Nelson[3], Analiza Alves[1], Lu Liu[2], Gusui Wu[1]; [1]DuPont Pioneer, Johnston, IA, U.S.A; [2]DuPont Pioneer, Hayward, CA, U.S.A.; [3]DuPont, Wilmington, DE, U.S.A..

14:15 **10 Discovery and optimization of hemipteran-active proteins for Lygus control in cotton** James A. Baum, Waseem Akbar, Konasale Anilkumar, David Bowen, Robert S. Brown, Cathy Chay, Thomas Clark, Michael Pleau, Xiaohong Shi, Uma Sukuru, Moritz Von Rechenberg, Halong Vu, Brent Werner, Andrew Wollacott; Monsanto Company, Chesterfield, Missouri U.S.A.

14:30 **11 Isolation and identification of potential biological control agent from *Tortrix viridana* L.(Lepidoptera: Tortricidae) pupae** Nurcan Albayrak Iskender[1]; Yaşar Aksu[2]; [1]Artvin Coruh University, Faculty of Arts and Sciences, Department of Biology, Artvin,Turkey; [2]Artvin Regional Forestry Management, Artvin, Turkey

14:45 **12 STU Evolution of a Sensor Protein Controlling Production of an Insecticidal Toxin in Plant-Beneficial *Pseudomonas protegens*** Peter Kupferschmied[1], Maria Péchy-Tarr[1], Nicola Imperiali[1], Monika Maurhofer[2], Christoph Keel[1]; [1]Department of Fundamental Microbiology, University of Lausanne, Switzerland; [2]Plant Pathology, Institute of Integrative Biology, ETH Zürich, Switzerland

15:00 **13 STU *Paenibacillus larvae*, the etiological agent of American Foulbrood, produces the catechol type siderophore bacillibactin** Gillian Hertlein[1]; Sebastian Müller[2]; Eva Garcia-Gonzalez[1]; Roderich D. Süßmuth[2]; Elke Genersch[1,3]; [1]Institute for Bee Research Hohen Neuendorf, Germany; [2]Technische Universität Berlin, Institut für Chemie, Berlin, Germany; [3]Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany

15:15 **14 Two new *Bacillus thuringiensis* toxins active against Lepidoptera and Coleoptera** Mikel Domínguez[1], Iñigo Ruiz de Escudero[1,2], Isabel Matas[2], Leopoldo Palma[1,2], Delia Muñoz[2], Primitivo Caballero[1,2], [1]Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain.[2]Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, Pamplona, Spain

15:30 **15-STU Entomopathogenic *Bacillus thuringiensis* as PGPR** Jiaheling Qi [1,2]; Daigo Aiuchi [2]; Shin-ichiro Asano[3]; Masanori Koike[2], [1]The United Graduate School of Agricultural Sciences, Iwate University, Iwate Japan; [2]Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan.;[3] Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

15:45 **16 Vibrios pathogenic for oysters are found associated to plankton species. What possible consequences on pathogen transmission to oysters?** Carmen Lopez-Joven[1], Jean-Luc Rolland [1]*, Eric Abaddie [2], Mohamed Laabir[1], Estelle Masseret[1], Audrey Vanhove[1], Audrey Caro[1], Delphine Bonnet[1], Delphine Destoumieux-Garzón[1]; [1]Ecology of coastal marine systems, UMR 5119, CNRS, Ifremer, IRD, University of Montpellier, France; [2]Laboratoire Environnement Ressource du Languedoc Roussillon, Ifremer, Sète, France.

Contributed Papers Monday, 14:00-16:00. **P1**

VIRUSES 1

Moderator: Eric Carstens and David Theilmann

14:00 **17 Investigation of Baculovirus RNA Polymerase Subunit Protein-Protein Interactions with *in vivo* Bimolecular Fluorescence Complementation Assays** Jessica Breznik, Nicola Johnson, Mustapha El-Ayoubi and Eric B Carstens, Queen's University, Kingston, Canada

14:15 **18 STU Characterization and Quantitative Analysis of *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV) FP25K Localization and Aggregate Formation During Cell Infection** Tyler A. Garretson and Xiao-Wen Cheng, Department of Microbiology, Miami University, Oxford, Ohio, USA

14:30 **19 STU Bracovirus-derived genes in the genome of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) and their role in host susceptibility to pathogens** Laila Gasmi, Agata K. Jakubowska, Juan Ferré, Salvador Herrero; Laboratory of Biochemical Genetics and Biotechnology, Department of Genetics, Universitat de València 46100 –Burjassot (Valencia), Spain

14:45 **20 Entry of *Bombyx mori* nucleopolyhedrovirus (BmNPV) into BmN Cells by Macropinocytic Endocytosis**, Jinshan Huang[1,2], Bifang Hao[1,2], Chen Cheng[1], Fei Liang[1], Xingjia Shen[1,2]; [1]Sericultural Research Institute, Jiangsu University of Science and Technology, [2]Sericultural Research Institute, Chinese Academy of Agricultural Science, Zhenjiang, Jiangsu, PRChina

15:00 **21 Nuclear translocation of *Autographa californica* nucleopolyhedrovirus ME53** Yang Liu, Jondavid de Jong, Éva Nagy, Peter Krell, University of Guelph, Guelph Ontario, Canada

15:15 **22 Nuclear localization and other domains of *Autographa californica* nucleopolyhedrovirus DNA polymerase** Guozhong Feng[1], Peter Krell[2], [1]State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, 310006, China; [2]University of Guelph, Guelph Ontario, Canada

15:30 **23 STU Investigations into the role of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) AC141 (EXON0) and *Trichoplusia ni* kinesin-1 in budded virus nucleocapsid egress** Siddhartha Biswas[1]; Gary W. Blissard[2]; David A. Theilmann[3], [1]Plant Science, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC Canada; [2]Boyce Thompson Institute at Cornell University, Ithaca, NY, USA; [3]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland BC, Canada

15:45 **24 The Twist In Baculoviruses** Loy Volkman, University of California, Berkeley, California, and Expression Systems, LLC, Davis, California, USA

Contributed Papers Monday, 14:00-15:30. **P2**

FUNGI 1

Moderators: Italo Delalibera and Nina Jenkins

14:00 **25 A new mycopesticide developed especially for the control of the citrus greening vector *Diaphorina citri* (Hemiptera: Liviidae)** Italo Delalibera Jr., Celeste P. D'Alessandro, Marcos R. Conceschi, John J. S. Ausique Department of Entomology and Acarology, ESALQ, University of São Paulo, Piracicaba, São Paulo, Brazil

14:15 **26 Effectiveness of biorationals and *B. bassiana* against tomato fruitworm in Sinaloa** Cipriano García, Adolfo D. Armenta and Luis A. Gaxiola; Instituto Politécnico Nacional. CIIDIR-IPN Unidad Sinaloa, Guasave, Sinaloa, Mexico

14:30 **27 Evaluating *Metarhizium brunneum* F52 Microsclerotia Applied in Hydromulch for Control of Asian Longhorned Beetles** Tarryn Anne Goble[1*], Ann Hajek[1], Mark Jackson[2], and Sana Gardescu[1]; [1]Department of Entomology, Cornell University, Ithaca, USA, [2]USDA-ARS-NCAUR, Crop Bioprotection Research Unit, Peoria, IL, USA

14:45 **28 STU Management of entomopathogenic fungal disease in rearing mealworms, *Tenebrio molitor* as animal feed** Sihyeon Kim, Se Jin Lee, Jeong Seon Yu, Yu-Shin Nai and Jae Su Kim; Department of Agricultural Biology, College of Agricultural & Life Sciences, Chonbuk National University, Jeonju, Korea

15:00 **29 Use of *Beauveria bassiana* (Bals) in the management of larger grain borer, *Prostephanus truncatus* (Horn.) (Coleoptera: Bostrichidae) on stored maize in Tanzania** Daniel Karanja[1], Pierre Grammare[2], Olivier Potin[2], Nick Jessop[3], Mathew Smith[3], Roger Day[1] and Belinda Luke[4] , [1]CABI Africa, Nairobi, Kenya, [2]SylvanBio, Société SOMYCEL SA, Loches, France, [3]Exosect Limited, Leylands Business Park, Colden Common, Hampshire, UK, [4]CABI Europe – UK, Egham, UK

15:15 **30 Management of *Frankliniella occidentalis* (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi** Jae Su Kim[1], Margaret Skinner[2], Bruce L. Parker[2], Se Jin Lee[1], Jeong Seon Yu[1] and Si Hyeon Kim[1], [1]Department of Agricultural Biology, College of Agricultural & Life Sciences, Chonbuk National University, Jeonju, Korea. [2]Entomology Research Laboratory, University of Vermont, Burlington, USA.

16:00–16:30 **BREAK**

Symposium 2 (Microsporidia) Monday, 16:30-18:30. **P3**

Microsporidiology: Advances in Europe

Organizers/Moderators: Andreas Linde and Sebastian Gisder

16:30 **31 A new intracellular parasite is a missing link between fungi and microsporidia** Karen L. Haag[1], Timothy Y. James[2], Ronny Larsson[3], Tobias M. M. Schaer[4], Dominik Refardt[5], Dieter Ebert[4]; [1]Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil; [2]University of Michigan, Ann Arbor, NI, USA; [3]University of Lund, Lund, Sweden; [4]Basel University, Basel, Switzerland; [5]Zurich University of Applied Sciences, Campus Grüental, Wädenswil, Switzerland

17:00 **32 Parasite takes fly - A Drosophila model of Microsporidia infection** Sebastian Niehus[1], Adrien Franchet[1], Frédéric Delbac[2], Michael Boutros[3], Dominique Ferrrandon[1]; [1]Institut de Biologie Moléculaire et Cellulaire, UPR 9022 du CNRS, Université de Strasbourg, Strasbourg, France; [2]Laboratoire Microorganismes: Génome et Environnement, UMR 6023 du CNRS, Université Blaise Pascal, Aubière, France; [3]German Cancer Research Center, Division of Signaling and Functional Genomics, and Department for Cell and Molecular Biology, Faculty of Medicine Mannheim, University of Heidelberg, Heidelberg, Germany

17:30 **33 White Sea metchnikovellids: morphology, life cycles; potential ancestral features of microsporidia** Yuliya Y. Sokolova[1,2]; [1]Core Microscopy Center, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, USA; [2]Institute of Cytology, St. Petersburg, Russia.

18:00 **34 Microsporidia: Pathogens of Opportunity** James J. Becnel[1], Louis M. Weiss[2]; [1]Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL 32608, USA, [2]Department of Pathology, Division of Parasitology and Tropical Medicine, and Department of Medicine Division of Infectious Diseases, Albert Einstein College of Medicine, Bronx, NY, USA

Contributed Papers Monday, 16:30-18:30. **P4**

NEMATODES 1

Moderators: Edwin Lewis and Albrecht Koppenhöfer

16:30 **35 Measuring entomopathogenic nematode activity, abundance and soil food web assemblage in Swiss wheat and maize cultivation** Raquel Campos-Herrera[1], Geoffrey Jaffuel[1], Xavier Chiriboga[1], Rubén Blanco-Pérez[1], Marie Fesselet[2], Vladimir Půža[3], Fabio Mascher[2], Ted C.J. Turlings[1]; [1]FARCE Laboratory, University of Neuchâtel, Neuchâtel (Switzerland); [2]Département fédéral de l'économie, de la formation et de la recherche DEFR, Agroscope, Institut des Sciences en Production Végétale IPV, Nyon (Switzerland); [3]Laboratory of Entomopathogenic Nematodes, Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

16:45 **36 STU Biocontrol and nutrition: understanding the role of environment in the trait deterioration of an entomopathogenic nematode symbiont** Dana Blackburn, Burke Crawford, and Byron Adams, Brigham Young University, Provo, UT, USA

17:00 **37 Insect-killing nematodes also kill competitors: lethal male-male fighting in Steinernema** Annemie Zenner, Kathryn O'Callaghan and Christine Griffin, Department of Biology, National University of Ireland Maynooth, Ireland

17:15 **38 STU Comparison of Life History Traits of the Entomopathogenic Nematodes *Steinernema feltiae* and *Steinernema riobrave*** Temesgen Addis[1,3], Asmamaw Teshome[2], Olaf Strauch[3] and Ralf-Udo Ehlers[3], [1]Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University, Kiel, Germany, [2]Department of Biology, Ghent University, Ghent, Belgium, [3]e~nema, GmbH, Schwentinental, Germany

17:30 **39 STU How does plant domestication influence entomopathogenic nematodes as potential biological control agents?** Monique Rivera[1], Cesar Rodriguez-Saona[1], Hans T. Alborn[2], and Albrecht M. Koppenhöfer[1], [1]Department of Entomology, Rutgers University, New Brunswick, NJ 08901, USA, [2]USDA ARS CMAVE, Gainesville, FL, USA

17:45 **40 Analysis of intraspecific variability in *Steinernema kraussei* populations using PCA**, M. Clausi[1], G. Rappazzo[1], E. Tarasco[2], D. Leone[1], M. T.Vinciguerra[1]; [1]Department of Biological, Geological and Environmental Sciences, Section of Animal Biology "M. La Greca", University of Catania, Catania (Italy), [2]Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari "Aldo Moro", Bari, Italy

18:00 **41 Population genetic structure of entomo-pathogenic nematode *Steinernema affine* (Steinernematidae: Nematoda) inferred using microsatellite markers** Vladimír Půža[1], Martina Žurovcová[1], Jiří Nermut'[1], Daniela Chundelová [1,2], Zdeněk Mráček[1]; [1]Institute of Entomology, Biology Centre of the AS CR, České Budějovice, Czech Republic; [2]Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic

18:15 **42 STU Eat or Be Eaten: Fungus and Nematode Switch off as Predator and Prey** E. Erin Morris[1] and Ann E. Hajek[2], [1]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg 1871, Denmark; [2]Department of Entomology, Cornell University, Ithaca, New York 14853-2601, USA

Contributed Papers Monday, 16:30-18:30. **P1**

VIRUSES 2

Moderators: Jenny Cory and Agata Jakubowska

16:30 **43 Insect feeding induces transgenerational resistance to NPV in Lepidoptera** Grant L. Olson[1], Judith H. Myers[2], Jenny S. Cory[1], [1]Dept. of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada; [2]Biodiversity Centre, Dept. of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

16:45 **44 The resistance of *Cydia pomonella* against baculoviruses is provoked by a mutation of the immediate-early *pe38* gene of *Cydia pomonell*a granulovirus** Manuela Gebhardt, Karolin E. Eberle, Johannes A. Jehle, Institute for Biological Control, Julius Kühn Institute (JKI), Federal Research Center on Cultivated Plants, Darmstadt, Germany

17:00 **45 CpGV-R5 allows replication of CpGV-M in resistant host insect larvae** Benoit Graillot[1,2], Sandrine Bayle[1], Christine Blachere-Lopez[1,3], Samantha Besse[2], Myriam Siegwart[4], Miguel Lopez-Ferber[1], [1]LGEI, Ecole des Mines d'Alès, Institut Mines-Telecom et Université de Montpellier Sud de France, Alès, France. [2]Natural Plant Protection, Arysta LifeScience group, Pau, France. [3]INRA, Alès, France. [4]INRA, unité PSH, AVIGNON, France

17:15 **46 Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*** Agata K. Jakubowska[1]; Melania D´Angiolo[1]; Rosa M. González Martínez[1]; Anabel Millán Leiva[1]; Arkaitz Carballo[2]; Rosa Murillo[2]; Primitivo Caballero[2]; Salvador Herrero[1], [1]Department of Genetics, Universitat de València, Burjassot, Spain; [2]Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Navarra, Spain

17:30 **47 Mixed SeMNPV genotypes comprised transmission capacities and insecticidal properties** Cristina Virto[1], David Navarro[1,2] Mª del Mar Tellez[2], Trevor Williams[3], Rosa Murillo[1,4], Primitivo Caballero[1,4], [1]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Ctra. de Mutilva s/n 31192, Mutilva Baja, Spain; [2]IFAPA, La Mojonera, 04745, Almería, Spain; [3]Instituto de Ecología AC, Xalapa 91070, Mexico; [4]Departamento Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain

17:45 **48-STU A novel mode of resistance of codling moth against *Cydia pomonella granulovirus*** Annette J. Sauer, Eva Fritsch, Karin Undorf-Spahn, Johannes A. Jehle, Julius Kühn-Institut, Darmstadt, Germany

18:00 **49 The effects of temperature on *Cryptophlebia leucotreta* granulovirus (GrleGV-SA) in mortality rates of false codling moth larvae *Thaumatotibia leucotreta*** Devon Brits, Jaryd Ridgeway & Alicia Timm, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa

18:15 **50 Enhancement of insecticidal activity of a nucleopolyhedrovirus isolated from *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) by coinfection with granulovirus** Paola Cuartas, Laura Villamizar; Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia

Contributed Papers Monday, 16:30-18:30. **P2**

FUNGI 2

Moderator: Drauzio Rangel

16:30 **51 Rapid and simple method for overnight development of strain-specific markers: A case study with the commercial *Beauveria bassiana* strain, GHA** George Kyei-Poku, Shajahan Johny, Agathe Roucou and Debbie Gauthier; Canadian Forestry Service, Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, Ontario, Canada

16:45 **52-STU The functions of two Cu/Zn-superoxide dismutases and a Fe-superoxide dismutase in regulating the growth, antioxidation, UV tolerance and virulence of *Beauveria bassiana*** Fang Li[1], Zheng-Liang Wang[2], Han-Qing Shi[1], Sheng-Hua Ying[1], Ming-Guang Feng[1], [1]Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China; [2]College of Life Sciences, China Jiliang University, Hangzhou, Zhejiang, P. R.China.

17:00 **53 STU Effect of temperature, water activity and UV-B radiation on conidia germination and colony growth of *Beauveria bassiana* isolates from soil and phylloplane** María Fernández-Bravo, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga, University of Córdoba, Department of Agricultural and Forestry Sciences, ETSIAM, 14071 Córdoba, Spain

17:15 **54 Non-target aquatic arthropods testing of *Metarhizium* strains and their crude extracts produced by solvent extraction and nanofiltration technology** Inmaculada Garrido-Jurado[1], Steffan R. Williams[2], Ahmed Abdrahman[3], Darren L. Oatley-Radcliffe[2], Enrique Quesada-Moraga[1], Tariq M. Butt[3]; [1]Department of Agricultural and Forestry Sciences, ETSIAM, University of Cordoba. Campus de Rabanales. Edificio C4 Celestino Mutis. 14071 Cordoba, Spain, [2]Centre for Water Advanced Technologies and Environmental Research (CWATER), College of Engineering, Swansea University, Swansea, UK, [3]Department of Biosciences, College of Science, Swansea University, Swansea, UK

17:30 **56 STU Development of analytical methods for the analysis of *Metarhizium brunneum* metabolites in crop matrices** Judith Taibon[1,2], Sonja Sturm[1], Christoph Seger[1,3], Hermann Stuppner[1], Hermann Strasser[2], [1]Institute of Pharmacy / Pharmacognosy, Leopold-Franzens University Innsbruck, Austria, [2]Institute of Microbiology, Leopold-Franzens University Innsbruck, Austria, [3]ZIMCL, University Hospital Innsbruck, Austria.

17:45 **57 STU α-1, 2-mannosyltransferase ktr1, ktr4 and kre2 regulate positively growth, conidiation, viability, virulence, and multi-stress tolerances in *Beauveria bassiana*** Juan-juan Wang, Lei Qiu, Sheng-Hua Ying, Ming-Guang Feng*, Institute of Microbiology, College of Life Sciences, Zhejiang Univ., Hangzhou, Zhejiang, People's Republic of China

SIP Division Business Meetings: Monday, 20:00-21:30

Microbial Control **P3**

DBI **P5**

Nematode Division Workshop Monday, 20:00-21:30 **P4**

Invertebrate Pathogens in the Classroom: Current Status and Future Challenges

Organizers: Glen Stevens and Patricia Stock

TUESDAY - 5 August

07:30-13:00 Registration **P1**

Symposium 3 (Fungi) Tuesday, 8:00-10:00. **P2**

Fatal Attraction: Fungi and Odours in deadly Combinations for Pest Control

Organizer/Moderator: Ingeborg Klingen

8:00 **58 Conifer - bark beetle - fungus interactions** Tao Zhao[1], Paal Krokene[2], Anna-Karin Borg-Karlson[1]. [1]The Royal Institute of Technology, Department of Chemistry, Ecological Chemistry Group, Stockholm, Sweden; [2]Norweigian Forest and Landscape Institute, Ås, Norway

8:20 **59 Carbon dioxide as an orientation cue for western corn rootworm and wireworm larvae - implications for an attract and kill approach using entomopathogenic fungi** Mario Schumann[1]; Anant Patel[2]; Miriam Hanitzsch[2]; Stefan Vidal[1];.[1]Georg-August-Universität Göttingen, Department of Crop Sciences, Göttingen, Germany; [2]Fachhochschule Bielefeld, University of Applied Sciences, Department of Engineering and Mathematics, Bielefeld, Germany

8:40 **60 Different behavioral responses in specialist and generalist natural enemy interactions (predators and fungi) in a strawberry-mite pest system** Stine Kramer Jacobsen[1], Jørgen Eilenberg[1], Ingeborg Klingen[2], Lene Sigsgaard[1], [1]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark; [2]Norwegian Institute for Agricultural and Environmental Research (Bioforsk) Plant Health and Plant Protection Division, Norway.

9:00 **61-STU How *Fusarium graminearum* influences insect-plant interactions** Drakulic Jassy[1,2]; Bruce Toby[2], Ray Rumiana[1]; [1]Division of Plant and Crop Sciences, University of Nottingham, UK; [2]Rothamsted Research, Department of Biological Chemistry and Crop Protection, Harpenden, UK

9:20 **62 Plant-microorganism interactions that shape host-plant selection in the grapevine moth** Geir K. Knudsen[1], Ilaria Pertot[2], Marco Tasin[1,3]; [1]Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway; [2]Edmund Mach Foundation, 38010 San Michele all´Adige, Italy; [3]Integrated Plant Protection, Dep. of Crop Protection Biology, Swedish University of Agricultural Sciences, Sweden

9:40 **63 Effect of host plant on aphid susceptibility to the fungal pathogen *Pandora neoaphidis*** Cezary Tkaczuk[1]; Paresh A. Shah[2], Judith K. Pell[2,3], [1]Department of Plant Protection, Siedlce University, Siedlce, Poland; [2]Plant and Invertebrate Ecology Department (now AgroEcology Department), Rothamsted Research, Harpenden, UK; [3]Current Address: J.K. Pell Consulting, Luton, UK

Contributed Papers Tuesday, 8:00-10:00. **P4**

NEMATODES 2

Moderators: Patricia Stock and Christine Griffin

8:00 **64 Entomopathogenic nematode behavioral responses to chemical cues from cadavers** Paige Redifer, Brittany Gale, Allison McLain, Glen Stevens, Laura Grochowski, School of Natural Sciences and Mathematics, Ferrum College, Ferrum, VA, USA

8:15 **65 The *Wolbachia* Endosymbiont as a Nematode Drug Target for Control of Human Filariasis, a Neglected Tropical Disease and Other insect Borne Pathogens** Barton E. Slatko, Molecular Parasitology Group. Genome Biology Division, New England Biolabs, Inc., Ipswich MA USA

8:30 **66 Differential PirAB expression of the entomopathogenic bacterium *Photorhabdus luminescens* (Enterobacteriaceae) based on tissue association and portal of entry to the insect host** Anaïs Castagnola[1,2]; Nathaniel Davis[3]; Belen Molina[4]; S. Patricia Stock[1]; John G. McMullen II[1]; [1]Department of Entomology, University of Arizona; [2]Center for Insect Science, University of Arizona; [3]Pima Community College; [4]Department of Ecology and Evolutionary Biology, University of Arizona, USA

8:45 **67-STU Candidate Virulence Loci in Pan-Genome of the Entomopathogenic Bacterium, *Xenorhabdus bovienii* (Gamma-Proteobacteria: Enterobacteriaceae),** John G McMullen II[1]; Gaelle Bisch[2]; Jean-Claude Ogier[2]; Sylvie Pagés[2]; Sophie Gaudriault[2]; S. Patricia Stock[3], [1]University of Arizona, School of Animal and Comparative Biomedical Sciences, Tucson, AZ; [2]Université Montpellier II/INRA, UMR 1333 Laboratoire DGIMI, Montpellier, France; [3]University of Arizona, Department of Entomology, Tucson, AZ, USA

9:00 **69 Molecular mechanism of the nematicidal activity of *Photorhabdus luminescens* LN2 against *Heterorhabditis bacteriophora* H06 nematodes** Xuehong Qiu and Richou Han Guangdong, Entomological Institute, Guangzhou 510260, China

9:15 **70 Natural products from entomopathogenic bacteria: Understanding the interaction of bacteria, insects and nematodes** Helge B. Bode, Merck Stiftungsprofessur für Molekulare Biotechnologie, Fachbereich Biowissen-schaften, Goethe Universität Frankfurt, Germany

Contributed Papers Tuesday, 8:00-10:00. **P1**

VIRUSES 3

Moderators: Zhihong Hu and Trevor Williams

8:00 **71 Characterization and formulation of a Colombian isolate of *Erinnyis ello* granulovirus (L.) (Lepidoptera: Sphingidae)** Juliana Gómez[1], Gloria Barrera[1], Paola Cuartas[1], Carolina Ruiz[1], Adriana Santos[1], Liz Uribe[1], Guillermo León[2], Laura Villamizar[1]; [1]Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia, . [2]Centro de Investigación "La Libertad" Corpoica, Puerto López, Colombia

8:15 **72 PRODUCTION OF the *Cydia pomonella* granulovirus (CpGV) IN A HETEROLOGOUS HOST** C.B. Chambers[1], S.D. Moore[2,3], M.P. Hill[3] & C. Knox[4], [1]River Bioscience, PO Box 20388, Humewood 6013, Port Elizabeth, South Africa, [2]Citrus Research International, PO Box 20285, Humewood 6013, Port Elizabeth, South Africa, [3]Department of Zoology and Entomology, Rhodes University, PO Box 64, Grahamstown, South Africa, [4]Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, South Africa

8:30 **73 Post-translational cleavage of P74 of the Helicoverpa armigera single nucleopolyhedrovirus facilitates *per os* infection** Huachao Huang[1], Manli Wang[1], Xin Luo[1], Xi Wang[1], Basil M. Arif[2], Fei Deng[1], Hualin Wang[1], Zhihong Hu[1] , [1]State Key Laboratory of Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, PR China; [2]Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

8:45 **74-STU Isolation, genetic characterisation and evaluation of biological activity of a novel South African *Phthorimaea operculella* granulovirus (*Phop*GV)** Michael D. Jukes[1], Caroline M. Knox[1], Sean D. Moore[2] & Martin P. Hill[3], [1]Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140 South Africa, [2]Citrus Research International, Humewood, Port Elizabeth, 6013 South Africa, [3]Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa

9:00 **75 Genetic and biological characterisation of a novel South African Plutella xylostella granulovirus, PlxyGV-SA** Fatima Abdulkadir[1], Caroline Knox[1] ,Tamryn Marsberg[2], Martin P. Hill[2] & Sean D. Moore[2,3], [1]Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, South Africa; [2]Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa; [3]Citrus Research International, Humewood, Port Elizabeth, South Africa

9:15 **76-STU Comparative transcriptome analysis of CpGV-M in susceptible and resistant codling moth *Cydia pomonella*** Diana Schneider, Johannes A. Jehle; Julius Kühn-Institut, Institute for Biological Control, Darmstadt, Germany

9:30 **77 Transmission of mixtures of insect pathogenic viruses in a single virion: towards the development of custom designed virus insecticides** Inés Beperet[1], Oihane Simón[1], Trevor Williams[2], Miguel López-Ferber[3], Primitivo Caballero[1]; [1]Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva Baja, Navarra, Spain; [2]Instituto de Ecologia AC, Xalapa, Mexico; [3]LGEI, École des Mines d'Alès, Alès France; [4]Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain

9:45 **78 Improvement of UV-resistance of Baculovirus by displaying the Nano-material binding peptides on the Polyhedron Envelope,** Jin Li, Yin Zhou, Chengfeng Lei, Xiulian Sun, Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

Contributed Papers Tuesday, 8:00-10:00. **P5**

BACTERIA 2

Moderators: Jean-Loius Schwartz and Juan Ferré

8:00 **79 *Yersina entomophaga* MH96 (Enterobacteriaceae) BC subcomplex of the Yen-Tc ABC toxin is able to induce toxicity independent of the A subcomplex** Sean D.G. Marshall[1], Jason N. Busby[2], J. Shaun Lott[2], Sandra A. Jones[1], Julie E. Dalziel[3], Femke Schepers[3], Mark Hurst[1]; [1]Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch 8140, New Zealand; [2]School of Biological Sciences, University of Auckland, New Zealand; [3]Food & Bio-based Products, AgResearch, Grasslands Research Centre, Palmerston North 4442, New Zealand

8:15 **80 Interaction of *Bacillus thuringiensis* Cry1Ab toxin with Mucus-rich structures** Diego Segond[1,2], Agnès Rejasse[1], Christophe Buisson[1], Shuyuan Guo[1,3], Karine Adel-Patient[2,4] , Hervé Bernard[2,4], Didier Lereclus[1], Christina Nielsen-LeRoux[1]; [1]INRA UMR1319-Micalis, team GME, 78352 Jouy en Josas France, [2]INRA, UR496 Unité d'Immuno-Allergie Alimentaire, France, [3]School of Life Science, Beijing Institute of Technology, Beijing, China, [4]CEA, IBiTecS, Service de Pharmacologie et d'Immunoanalyse, Gif-sur-Yvette, France

8:30 **81-STU Pore formation helping ability and binding affinity of BmABCC2 and BtR175 against Cry1A toxins** Shiho Tanaka[1]; Ami Iizuka[1]; Kazuhisa Miyamoto[2]; Hiroaki Noda[2]; Shingo Kikuta[1]; Ryoichi Sato[1]; [1]Tokyo University of Agriculture and Technology, Koganei,Tokyo, Japan; [2]National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan

8:45 **82 A necessary step in the mode of action of the Cry8 toxin: the elimination of DNA from the Cry toxin-DNA complex,** Shuyuan Guo, Bingjie Ai, Jie Li, Dongmei Feng, Feng Li, School of Life Science, Beijing Institute of Technology, Beijing, China

9:00 **83-STU How does the *Bt* Cry41Aa toxin kill human cancer cells?** Barbara Domanska, Vidisha Krishnan, Gizem Altun, Michelle West and Neil Crickmore; Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, UK

9:15 **84-STU Which regions of the *Bt* Cry41Aa toxin are responsible for its activity against human cancer cells?** Alicia Elhigazi, Vidisha Krishnan, Fatai Afolabi, Barbara Domanska, Lisa Muharib, Michelle West, Neil Crickmore. Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, UK

9:30 **85 Parasporin PS1Aa2 induces ionic channels in lipid bilayer membranes and calcium oscillations in sensitive cells** Gabriel Narvaez[1], Vincent Vachon[1], Dong Xu[2], Jean-Charles Côté[2], Jean-Louis Schwartz[1,3], [1]Groupe d'étude des protéines membranaires, Université de Montréal, Montreal, Quebec, Canada; [2]Research Center, Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada, [3]Centre Sève, Université de Sherbrooke, Sherbrooke, Quebec, Canada

9:45 **86-STU** ***Caenorhabditis elegans – Bacillus thuringiensis* interactions: new insights into mechanisms of host resistance and pathogen virulence** Igor Iatsenko, Iuliia Boichenko, Ralf J. Sommer; Max Planck Institute for Developmental Biology, Department for Evolutionary Biology, Tuebingen, Germany

10:00–10:30 **BREAK**

Symposium 4 (Viruses) Tuesday, 10:30-12:30. **P1**

Small non-coding RNAs as Regulators of Insect Host-Virus Interactions and Immunity

Organizer/Moderator: Sassan Asgari

10:30 **87 Role of cellular and virus-encoded microRNAs in insect host-virus interactions** Sassan Asgari, School of Biological Sciences, The University of Queensland, Brisbane QLD 4072, Australia

11:00 **88 Sensing viral RNA in *Drosophila melanogaster*** Simona Paro[1], Eric Aguiar[2], Bill Claydon[1], Joao Trindade Marques[2], Jean-Luc Imler[1,2] and Carine Meignin[1,2]; [1]IBMC, CNRS-UPR9022, Strasbourg, France; [2]Laboratory of RNA Interference, Biochemistry and Immunology, Universidade Federal de Minas Gerais Belo Horizonte, Brazil; [3]University of Strasbourg, Strasbourg, France

11:30 **89 Small RNA-directed antiviral immunity in disease-vector mosquitoes** Kevin M. Myles, Virginia Tech, Fralin Life Science Institute, Department of Entomology, Blacksburg, Virginia, USA

12:00 **90 Controlling viral infection in insects** Mark Kunitomi, Michel Tassetto, Arabinda Nayak, and Raul Andino, Department of Microbiology and Immunology, University of California, San Francisco, California 94143-2280, USA

Contributed Papers Tuesday, 10:30-12:15. **P3**

MICROBIAL CONTROL 1

Moderator: Michael Brownbridge

10:30 **91 Double trouble for thrips: Effective biopesticide combinations to control soil-dwelling stages in chrysanthemums** Michael Brownbridge, Taro Saito and Paul Côté, Vineland Research and Innovation Centre, Vineland Station, Ontario, Canada

10:45 **92-STU Lethal and sub-lethal impacts of fungal biopesticides on house fly populations in simulated field settings of biocosms,** Naworaj Acharya[1], Simon Blanford[1,2], Edwin G. Rajotte[1], Nina E. Jenkins[1], Mathew B. Thomas[1,2]; [1]Department of Entomology, Penn State University, 501 Agricultural Sciences and Industries Building, PA 16802, USA, [2]Center for Infectious Diseases Dynamics, Penn State University, Merkle Lab, PA 16801, USA

11:00 **93-STU Management of *Prostephanus truncatus* (Horn.) on stored maize using *Beauveria bassiana* (Bals.)** Mavis A. Acheampong[1], Eric W. Cornelius[1], Vincent Y. Eziah[1], Ken O.Fening[1], Clare Storm[2], Dave Moore [3], Nick Jessops[2], Matthew Smith[2], Olivier Potin[4], Pierre Grammare[4] and Belinda Luke[3]; [1]Department of Crop Science, University of Ghana, Legon; [2]Exosect Ltd, UK; [3]CABI, UK; [4]SylvanBio, France

11:15 **94-STU Lack of involvement of chitinase in direct toxicity of *Beauveria bassiana* exudates to the aphid *Myzus persicae*** Peter Cheong[1], Travis R. Glare[1], Michael Rostas[1], Stephen Haines[2], Jolon Dyer[2], Stefan Clerens[2], Jenny Brookes[1] and Stephen Ford[3]; [1]Bio-Protection Research Centre, P O Box 85084, Lincoln University, Lincoln 7647, Christchurch, New Zealand, [2]AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand, [3]Biotelliga Limited, Pukekohe 2120, New Zealand

11:30 **95-STU Entomopathogenic fungi for control of false codling moth in South African citrus orchards** Candice A. Coombes[1]; Martin P. Hill[1]; Sean D. Moore[1,2]; Joanna F. Dames[3], [1]Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140, South Africa; [2]Citrus Research International, Humewood, 6013, Port Elizabeth, South Africa; [3]Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140, South Africa.

11:45 **97-STU Wireworm control with entomopathogenic fungi and plant extracts** Sonja Eckard[1]; Sven Bacher[2]; Jürg Enkerli[1]; Giselher Grabenweger[1]; [1]Agroscope, Institute for Sustainability Sciences, Reckenholzstrasse 191, Zürich, Switzerland, [2]University of Fribourg, Department of Biology, Unit of Ecology and Evolution, Fribourg, Switzerland

12:00 **98-STU Long-term persistence of *Beauveria brongniartii* BIPESCO 2 used for cockchafer control in the Euroregion Tyrol** Johanna Mayerhofer[1,2], Jürg Enkerli[2], Roland Zelger[3] & Hermann Strasser[1]; [1]Institute of Microbiology, Leopold-Franzens University Innsbruck, AUT, [2]Molecular Ecology, Institute for Sustainability Sciences, Agroscope, Zürich, CH, [3]Research Centre for Agriculture and Forestry Laimburg, Ora/Auer, Italy

Contributed Papers Tuesday, 10:30-12:30. **P4**

DIS. OF BENEFICIAL INVERTEBRATES 1

Moderators: Kelly Bateman and Spencer Greenwood

10:30 **99 The Curious Case of the PaV1 in Adult Caribbean Spiny Lobsters** Donald C. Behringer[1,2]; Mark J. Butler IV[3]; Jessica Moss[4]; Jeffrey D. Shields[4]; [1]University of Florida, Program in Fisheries and Aquatic Sciences, Gainesville, Florida 32653 (USA); [2]University of Florida, Emerging Pathogens Institute, Gainesville, Florida 32611 (USA); [3]Old Dominion University, Department of Biological Sciences, Norfolk, Virginia 23529 (USA); [4]Virginia Institute of Marine Science, Gloucester Point, Virginia 23062 USA

10:45 **100 Defining lobster-pathogen interactions via high-throughput gene expression studies: The discovery and description of the interplay between the American Lobster (*Homarus americanus*) and the ciliated parasite *Anophryoides haemophila,*** Spencer J. Greenwood[1,2]; K. Fraser Clark[1,2,3], [1]Atlantic Veterinary College Lobster Science Centre; [2]Department of Biomedical Sciences, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada; [3]Department of Plant and Animal Sciences, Dalhousie University, Truro, Nova Scotia, Canada

11:00 **101-STU Metabolomic investigation of Bitter Crab Disease in snow crabs (*Chionoecetes opilio*)** Melanie

Buote[1], Russ Kerr[2], Rick Cawthorn[1], Spencer Greenwood[2], Glenda Wright[2]; [1]Department of Pathology and Microbiology, Atlantic Veterinary College at UPEI, Charlottetown, PEI; [2]Department of Biomedical Sciences, Atlantic Veterinary College at UPEI, Charlottetown, PEI

11:15 **102-STU Assessment of immunocompetence in the shore crab, *Carcinus maenas*, to natural exposure of pathogens** Lauren Hall[1], Chris Hauton[1], Grant Stentiford[2], [1]National Oceanography Centre Southampton, University of Southampton, European Way, Southampton, SO14 3ZH, UK, [2]CEFAS, The Nothe, Barrack Road, Weymouth, Dorset, DT4 8UB, UK

11:30 **103-STU Effects of artificial infection of juvenile edible crabs, *Cancer pagurus* with the parasitic dinoflagellate, *Hematodinium* sp.** Amanda Smith, Andrew Rowley; Department of Biosciences, College of Science, Swansea University, Swansea, SA2 8PP, Wales, U.K.

11:45 **104 A role of polychaetes in transmission of white spot syndrome virus in shrimp ponds?** H. Desrina[1,2,3], Marc C.J. Verdegem[2], Johan A.J. Verreth[2], Slamet B. Prayitno[3] and Just M. Vlak[1]; Laboratories of [1]Virology and [2]Aquaculture and Fisheries, Wageningen University, Wageningen, The Netherlands, and [3]Department of Fisheries, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof Sudharto, Tembalang, Semarang, Indonesia.

12:00 **105 Novel Pattern Recognition Receptor Protects Shrimp from *Vibrio* Infection by Binding Flagellin and LPS through Different Recognition Modules,** Xian-Wei Wang; Jin-Xing Wang, School of Life Sciences, Shandong University, Jinan, China

12:15 **106 Observations on *Agmasoma penaei* and *Perezia nelsoni* in White shrimp *Litopenaeus setiferus* from the Gulf of Mexico** Yuliya Sokolova[1,3], John Hawke[2], [1]Core Microscopy Center, [2]Dept. Pathobiol.Sci., School Vet. Medicine, Louisiana State University, Baton Rouge LA, USA; [3]Institute of Cytology, St. Petersburg, Russia

Contributed Papers Tuesday, 10:30-12:15. **P2**

FUNGI 3

Moderators: Helen Hesketh and Ann Hajek

10:30 **107 Comparison of ecological traits of co-existing *Metarhizium*: What does it take to dominate an agricultural field?** Bernhardt M. Steinwender[1], Miriam Stock[2], Kasper Brink - Jensen[3], Jørgen Eilenberg[1], Nicolai V. Meyling[1], [1]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; [2]IST Austria (Institute of Science and Technology Austria), Klosterneuburg, Austria; [3]Department of Biostatistics, University of Copenhagen, Denmark

10:45 **108-STU Effect of entomopathogenic fungal strains on non-target arthropods in sour cherry orchard** Emese Balog, Zoltán István Tímár, Judit Papp-Komáromi, György Turóczi; Szent István University, Plant Protection Institute, Gödöllő, Hungary

11:00 **109-STU Potential of endophytic *Beauveria bassiana* in grapevine against insects** Yvonne Rondot, Annette Reineke, Hochschule Geisenheim University, Center of Applied Biology, Institute of Phytomedicine, Geisenheim, Germany

11:15 **111 Horizontal transmission of entomopathogenic fungi by ectoparasitoid *Habrobracon hebetor*** Vadim Kryukov, Natalia Kryukova, Olga Yaroslavtseva, Victor Glupov; Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

11:30 **112 N Fast spread of the parasitic *Laboulbenia formicarum* in a supercolony of the invasive garden ant *Lasius neglectus*** Simon Tragust[1], Heike Feldhaar[1], Jes Søe Pedersen[2]; [1]Animal Ecology I, University of Bayreuth, Germany, [2]Centre for Social Evolution, Department of Biology, University of Copenhagen, Denmark

11:45 **113 The dietary preference of a beneficial predator in apple orchards reveals an undocumented spore dispersal mechanism for entomopathogenic fungi** Anja Amtoft Wynns[1]; Annette Bruun Jensen[1], Celeste d'Allesandro[2], Jørgen Eilenberg[1]; [1]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; [2]Department of Entomology and Acarology, ESALQ, University of São Paulo, Brazil

12:00 **114 Effects of entomopathogenic fungi on the "*Trialeurodes vaporariorum* – *Encarsia formosa*" system: preliminary results** Monica Oreste, Eustachio Tarasco, Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari, Bari, Italy

12:40-16:30 Optional Excursion

16:30-18:00 5K Race

17:00-21:30 BBQ

WEDNESDAY - 6 August

7:30-18:00 **REGISTRATION** **P1**

Symposium 5 (Microbial Control) Wednesday, 8:00–10:00. **P3**

Developments/Issues in the Regulation of Microbial Products: Harmonization across Jurisdictions

Organizers/Moderators: Roma Gwynn and David Grzywacz

8:00 **115 The authorisation and regulation of microbial biopesticides: why bother?** David Chandler[1], Liam Harvey & Wyn Grant[2], [1]Warwick Crop Centre, School of Life Sciences, University of Warwick, UK, [2]Department of Politics and International Studies, University of Warwick, UK

8:24 **116 Registration of Biopesticides in the EU: a company perspective** Philip Kessler, Andermatt Biocontrol AG, Grossdietwil, Switzerland

8:48 **117 Biopesticide registration, a company perspective and how registration influences biopesticide R&D approach of companies in North American** Jarrod Leland, Novozymes Biologicals, Inc., 5400 Corporate Circle, Salem, United States

9:12 **118 Registration of biopesticides: how research can be structured to suit microbial registration needs and promote the commercial development of new biopesticides** Roma Gwynn, Biorationale Limited, Duns, UK

9:36 **119 Current developments and issues on regulation of biopesticides- Lessons from REBECA project, comparison of EU and USA systems** Sabine Asser-Kaiser, Jacqueline Süß, Rüdiger Hauschilld; GAB Consulting GmbH, Heidelberg/Lamstedt, Germany

Contributed Papers Wednesday, 8:00-9:45. **P5**

BACTERIA 3

Moderators: Juan Luis Jurat-Fuentes and David Heckel

8:00 **120 Resistance alleles to *Lysinibacillus sphaericus* are co-select in a *Culex quinquefasciatus* colony and display distinct features** Maria Helena N. L. Silva-Filha[1], Karlos D. M. Chalegre[1], Tatiany P. Romão[1], Daniella A. Tavares[1], Hervely S. G. Menezes[1], Cláudia M. F. de Oliveira[1], Osvaldo P. de-Melo-Neto[2], [1]Department of Entomology, [2]Department of Microbiology, Centro de Pesquisas Aggeu Magalhães-FIOCRUZ, Recife, Brazil

8:15 **121-STU Untangling insect pathogenicity in plant-beneficial pseudomonads by a combination of comparative genomics, bioassays and histopathology** Pascale Flury[1], Beat Ruffner [1], Shakira Fataar[1], Maria Péchy-Tarr[2], Regina G. Kleespies[3], Cornelia Ullrich[3], Johannes A. Jehle[3], Theo H. M. Smits[4], Christoph Keel[2], Monika Maurhofer[1], [1]Institute of Plant Pathology, Swiss Federal Institute of Technology, Zürich, Switzerland; [2]Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; [3]Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; [4]Research Group for Environmental Genomics and Systems Biology, Institute for Natural Resources Sciences, Zurich University of Applied Sciences ZHAW, Wädenswil, Switzerland

8:30 **122 Comparative analysis of the Cqm1 and Aam1 ortholog proteins from mosquitoes that have a differential capacity to bind to the Binary toxin from *Lysinibacillus sphaericus*** Lígia M. Ferreira[1], Nathaly A. do Nascimento[1], Tatiany P. Romão[1], Antônio M. Rezende[2], Osvaldo P. de-Melo-Neto[2], Maria Helena N. L. Silva-Filha[1], [1]Department of Entomology, [2]Department of Microbiology, Centro de Pesquisas Aggeu Magalhães-FIOCRUZ, Recife, Brazil

8:45 **123 Resilience of the intestinal epithelium to the action of a bacterial pore-forming toxin and to xenobiotics in Drosophila** Kwang-Zin Lee, Matthieu Lestradet, Stephanie Limmer, Samuel Liégeois and Dominique Ferrandon; University of Strasbourg Institute for Advanced Study, IBMC, Strasbourg, France

9:00 **124 Cadherin mutations and Bt resistance: Field screening and fitness costs** Linda Gahan[1]; Fred Gould[2], David G. Heckel[3]; [1]Clemson University, Clemson, South Carolina, USA; [2]North Carolina State University, Raleigh, North Carolina, USA; [3]Max Planck Institute for Chemical Ecology, Jena, Germany

9:15 **125 Down regulation and mutation of cadherin gene associated with Cry1Ac resistance in Asian corn borer** Tingting Jin[1], Xue Chang[1], Angharad M. R. Gatehouse[2], Zhenying Wang[1], Martin E. Edward [2], Kanglai He[1], [1]The State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China; [2]Newcastle Institute for Research on Environment and Sustainability, School of Biology, University of Newcastle, UK

9:30 **126 ABCC transporters mediate insect resistance to multiple Bt toxins revealed by BSA analysis** Youngjin Park[1], Rosa M González-Martínez[2], Gloria Navarro-Cerrillo[2], Maissa Chakroun[2], Yonggyun Kim[1], Peio Ziarsolo[3], Jose Blanca[3], Joaquin Cañizares[3], Juan Ferré[2], Salvador Herrero[2]; [1]Department of Bioresource Sciences, Andong National University, Korea, [2]Department of Genetics, Universitat de València, Spain, [3]Institute for Conservation & Improvement of Valentian Agrodiversity (COMAV).Polytechnic University of Valencia, Spain

Contributed Papers Wednesday, 8:15-9:45. **P4**

DIS. OF BENEFICIAL INVERTEBRATES 2

Moderator: Lena Poppinga

8:15 **128 *Nosema ceranae* News: Update on Species Competition and Host-Pathogen Interaction Studies** Leellen Solter[1], Zachary Huang[2], Wei-Fone Huang[1] and Meghan Milbrath[2]; [1]Illinois Natural History Survey, University of Illinois; [2]Michigan State University

8:30 **129 Influence of temperature on the development of *Nosema apis* and *Nosema ceranae*** Sebastian Gisder; Elke Genersch; Institute for Bee Research, Hohen Neuendorf, Germany

8:45 **130-STU The involvement of bumblebee small interfering RNA pathway against two different bee viruses** Jinzhi Niu, Ivan Meeus, Guy Smagghe; Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

9:00 **131 Impact of Wolbachia endosymbionts on the evolution of sex determination in the isopod Armadillidium vulgare** Sébastien Leclercq, Julien Thézé, Isabelle Giraud, Lise Ernenwein, Bouziane Moumen, Pierre Grève, Clément Gilbert, Richard Cordaux, Université de Poitiers, UMR CNRS 7267 Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Poitiers Cedex, France

9:15 **132 First characterization of a mollusk beta pore forming toxin** David Duval[1,2], Richard Galinier[1,2], Guillaume Mitta[1,2], Benjamin Gourbal[1,2]; [1]CNRS, UMR 5244, Ecologie et Evolution des Interactions (2EI), Perpignan, France, [2]Université de Perpignan, Perpignan, France

9:30 **133-STU A first report of an immune-associated cytosolic PLA_2 in insects: Gene structure and function** Jiyeong Park and Yonggyun Kim; Department of Bioresource Sciences, Andong National University, Andong, Korea

Contributed Papers Wednesday, 8:00-9:30 **P2**

FUNGI 4

Moderator: Richard Humber and Annette Brunn Jensen

8:00 **134 Fungal dimorphism in the entomopathogenic fungus *Nomuraea rileyi*: A search for *in vivo* produced quorum-sensing molecules** Boucias, Drion[1], Liu, Shouzou[2] and Baniszewski, Julie[1], [1]Entomology and Nematology Department, University of Florida, Gainesville FL, USA, [2]Agricultural College, Liaocheng University, Liaocheng, Shandong, China

8:15 **135 Multilocus genotyping of *Amylostereum* spp. associated with *Sirex noctilio* and other woodwasps from Europe reveal clonal lineage introduced to the US** Louela A. Castrillo[1], Ann E. Hajek[1], Ryan M. Kepler[1], Juan A. Pajares[2], Iben M. Thomsen[3] , György Csóka[4], Paula Zamora[5] , and Sergio P. Angeli[6], [1]Department of Entomology, Cornell University, Ithaca, USA, [2]Sustainable Forest Management Research Institute, University of Valladolid, Palencia, Spain, [3]Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark, [4]Department of Forest Protection, Forest Research Institute, Mátrafüred, Hungary, [5]Calabazanos Forest Health Center, Castile and Leon, Palencia, Spain, [6]Faculty of Science and Technology, University of Bolzano, Italy

8:30 **136 Preliminary analysis of the genome sequence of *Beauveria caledonica*** Travis R. Glare[1], Aimee C. McKinnon[1] and Murray P. Cox[2], [1]Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand, [2]Massey University, Palmerston North, New Zealand

8:45 **137 MALDI-TOF Mass Spectrometry: A complement to sequence-based identification technologies for major fungal entomopathogens** Richard A. Humber[1], Rogério Biaggioni Lopes[2], Marcos Faria[2]; [1]USDA-ARS Biological IPM Research, RW Holley Center, Ithaca, New York, USA; [2]Embrapa Genetic Resources and Biotechnology, Brasília, Brazil

9:00 **138 Transcriptomic study reveals *Pandora formicae* expressing pathogenicity related genes in final stages of host infection** Joanna Malagocka[1]; Morten N. Grell[2], Lene Lange[2], Jørgen Eilenberg[1], Annette Bruun Jensen[1]; [1]Centre for Social Evolution, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; [2]Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Copenhagen, Denmark

9:15 **139 Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals putative virulence factors shared by fungal and oomycete entomopathogens** Paula F. Quiroz Velasquez, Sumayyah Abiff, Quincy B. Conway, Norma C. Salazar, Ana Paula Delgado, Jhanelle K. Dawes, Lauren G. Douma, Aurélien Tartar; Nova Southeastern University, Fort Lauderdale, FL, USA

10:00–10:30 **BREAK**

Symposium 6 (Bacteria) Wednesday, 10:30-12:30. **P5**

Structure and Function of Novel Insecticidal Toxins

Organizers/Moderators: Ken Narva and Colin Berry

10:30 **140 Structural and biophysical characterization of Cry34Ab1 and Cry35Ab1** Matthew S. Kelker[1], Colin Berry[2], Matthew D. Baker[2], Steven L. Evans[1], Reetal Pai[1], David McCaskill[1], Joshua C. Russell[1‡], Nick X. Wang[1], J.W. Pflugrath[3], Cheng Yang[3], Matthew Wade[4], Tim J. Wess[4#], Kenneth E. Narva[1], [1]Dow AgroSciences, LLC, Indianapolis, Indiana, USA; [2]Cardiff School of Biosciences, Cardiff University, Cardiff, Wales, UK; [3]Rigaku Americas Corporation, The Woodlands, Texas, USA; [4]School of Optometry & Vision Sciences, Cardiff University, Cardiff, Wales, UK, [‡]Current address: Department of Biochemistry, University of Washington, Seattle, Washington, USA; [#]Current address: Office of the Dean of Science, Charles Sturt University, New South Wales, Victoria, Australia

10:50 **141 Structure/function studies of Cry5B via alanine-scanning mutagenesis** Jillian Sesar[1]; Melanie Miller[1], Yan Hu[1,2], Raffi V. Aroian[1,2], [1]Division of Biological Sciences, University of California, San Diego, CA, USA; [2]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA

11:10 **142 Insights into the structures of non-3-domain toxins through structural modelling** Colin Berry, Cardiff School of Biosciences, Cardiff Univ., Cardiff, UK

11:30 **143 Novel MTX Toxins for Insect Control** Yong Yin, Monsanto Company, St. Louis, MO, USA

11:50 **144 Insecticidal toxins from *Photorhabdus luminescens* and *asymbiotica*, targeting the actin cytoskeleton and GTP-binding proteins** Thomas Jank, Alexander E. Lang and Klaus Aktories; Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Freiburg, Germany

12:10 **145 Molecular basis of parasporin-2 action toward cancer cells** Sakae Kitada, Yusuke Yoshida, Yoshimi Ozaki, Hirioyasu Shimada, Kyushu Institute of Technology, Iizuka,

Contributed Papers Wednesday, 10:30-12:30. **P3**

MICROBIAL CONTROL 2

Moderator: Surrendra Dara

10:30 **146 Evaluation of the non-target effects of *Bacillus thuringiensis* subspecies *israelensis* in standardized aquatic microcosms** Irene Ketseoglou; Gustav Bouwer, School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa

10:45 **147 *Bacillus thuringiensis* 00-50-5 strain with high activity against plant-parasitic nematodes and insect pests** Cheng Bai[1], Haibo Long[1], Liping Liu[1], Yanling Yang[2], Jianjun Yue[1]; [1]Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan, China; [2]North University of China, Taiyuan, China

11:00 **148 Investigations on residues of *Bacillus thuringiensis* on tomato** Dietrich Stephan[1]; Heike Scholz-Döblin[2], Hans Kessler[2], Theo Reintges[2], [1]Julius Kühn-Institute, Darmstadt, Germany, [2]Landwirtschaftskammer Nordrhein-Westfalen, Germany

11:15 **149 Biological control of western corn rootworm larvae (*Diabrotica virgifera virgifera*) with Dianem® (*Heterorhabditis bacteriophora*)** Ralf-Udo Ehlers, e~nema, GmbH, Schwentinental, Germany

11:30 **150 Evaluation of Ten Plant Extracts as Ultraviolet Protectants for *Spodoptera littoralis* nucleopolyhedrovirus** Koko Dwi Sutanto, Said El Salamouny, Martin Shapiro, Merle Shepard, Sukirno Miharjo, Muhammad Tufail, Khawaja Ghulam Rasool and Abdulrahman S. Aldawood, Plant Protection Department, College of Food Sciences and Agriculture, King Saud University, Riyadh, Saudi Arabia; CREC, Clemson University, Charleston South Carolina, USA

11:45 **151 Interactions among Fungal and Viral Pathogens and Parasitoids** Ann E. Hajek[1]; Saskya van Nouhuys[2], [1]Department of Entomology, Cornell University, Ithaca New York, USA, [2]Department of Biosciences, University of Helsinki, Helsinki, Finland

12:00 **152 *Oryctes rhinoceros* population diversity and potential implications for control using *Oryctes nudivirus*** Sean D.G. Marshall[1], Aubrey Moore[2], Russell K. Campbell[3], Roland J. Quitugua[2], Trevor A. Jackson[1], [1]Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch, New Zealand; [2]College of Natural and Applied Science, University of Guam, USA; [3]Biosecurity Division, Guam Department of Agriculture, Guam, USA

12:15 **153 The Control of Fungi Using with Liposomal Formulation of Essential Oil of *Satureja hortensis* and its cell viability assay** Müge Yazici[1], Gülengül Duman[2], Ismail Aslan[2],Burçin Asutay[1], Tuğçe Palamut[1], Sıdıka Tapşın[1], Fikrettin Şahin[1], [1]Department of Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey, [2]Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey

Contributed Papers Wednesday, 10:30-12:15. **P1**

VIRUSES 4

Moderators: Martin Erlandson and Robert Harrison

10:30 **154 *Mamestra configurata* nucleopolyhedrovirus-A transcriptome from infected host midgut** Martin A. Erlandson[1], B. Cameron Donly[2], David A. Theilmann[3], Dwayne D. Hegedus[1], Cathy Coutu[1] and Douglas Baldwin[1], [1]Saskatoon Research Centre, AAFC, Saskatoon, Canada; [2]Southern Crop Protection & Food Research Centre, AAFC, London, Canada; [3]Pacific Agri-Food Research Centre, AAFC, Summerland, BC, Canada

10:45 **155-STU Genomic adaptation to different hosts – Impact of genetic diversity on viral fitness** Aurélien Chateigner; Cindy Pontleve; Carole Labrousse; Elisabeth Herniou, Institut de Recherche sur la Biologie de l'Insecte, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Tours, France

11:00 **156-STU Transcriptomic analysis of a host-parasitoid interaction between a Hymenoptera *Cotesia congregata*, a Lepidoptera *Manduca sexta* and a Polydnaviridae** Germain Chevignon; Sébastien Cambier; Jean-Michel Drezen; Elisabeth Huguet; Sébastien Moreau; Institut de Recherche sur la Biologie de l'Insecte, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Tours, France

11:15 **157 Expressed viral ORF and new virus discovery from high throughput transcriptomes of non-model animal** Diane Bigot[1], Marion Ballenghien[2], Vincent Cahais[2], Nicolas Galtier[2], Elisabeth Herniou[1], Philippe Gayral[1], [1]Institut de Recherches sur la Biologie de l'Insecte, CNRS UMR 7261, Université François-Rabelais, Tours, France. [2]Université Montpellier 2, Institut des Sciences de l'Evolution de Montpellier, Montpellier, France

11:30 **158 Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons** Clément Gilbert[1], Aurélien Chateigner[2], Lise Ernenwein[1], Valérie Barbe[3], Annie Bézier[2], Elisabeth A. Herniou[2,*] & Richard Cordaux[1], [1]Université de Poitiers, Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Poitiers Cedex, France; [2]Université François-Rabelais de Tours, Tours, France, [3]Laboratoire de Finition, CEA/IG/Genoscope, Evry, France

11:45 **159 Genomic analysis of five *Lymantria dispar multiple nucleopolyhedrovirus* isolates and biological activity against different host strains of *Lymantria dispar*** Robert L. Harrison[1]; Daniel L. Rowley[1]; Melody Keena[2], [1]Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, USDA Agricultural Research Service, Beltsville, Maryland, USA; [2]Northern Research Station, USDA Forest Service, Hamden, CT, USA

12:00 **160 Phylogenomics reveals ecological factors that lead to speciation in *Baculoviridae*** Julien Thézé[1]; Carlos Lopez Vaamonde[2]; Jennifer S. Cory[3]; Elisabeth A Herniou[1], [1]Université François-Rabelais, UFR Sciences, Tours, France; [2]INRA, Zoologie Forestière, Orléans, France; [3]Dept of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

Contributed Papers Wednesday, 10:30-12:15. **P2**

FUNGI 5

Moderators: Travis Glare and Jürg Enkerli

10:30 **162 An entomopathogenic strain of *Beauveria bassiana* against *Frankliniella occidentalis* with no detrimental effect on the predatory mite *Neoseiulus barkeri*** Yulin Gao[1], Shengyong Wu[1], Zhongren Lei[1], Xuenong Xu[1], [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

10:45 **163-STU Interactions between the insect pathogenic fungus *Metarhizium*, the wheat pathogen *Fusarium culmorum* and the mycoparasitic fungus *Clonostachys rosea*** Chad A. Keyser, Birgit Jensen, and Nicolai V. Meyling, Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

11:00 **164 Diversity, ecology and virulence of entomopathogenic fungi isolates naturally infecting the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the Mediterranean Basin** Natalia González-Mas, Lola Ortega-García, Carlos Campos-Porcuna, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga; University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain

11:15 **165-STU Recovery and detection of an entomo-pathogenic endophyte: overcoming the challenges involved** Aimee McKinnon[1]; Travis Glare[1], Hayley Ridgway [2], Andrew Holyoake[1], [1]Bio-Protection Research Centre, Lincoln University, Christchurch, New Zealand; [2]Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand

11:30 **166-STU Intense spatio temporal pattern in pathogen-host interaction between *Pandora formicae* and *Formica rufa*** Joanna Malagocka; Jørgen Eilenberg, Annette Bruun Jensen; Centre for Social Evolution, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark

11:45 **167 Patterns of host adaptation in fly infecting *Entomophthora* species** Henrik H. De Fine Licht; Annette Bruun Jensen, Jørgen Eilenberg, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

12:00 **168-STU Plant volatile organic compound manipulation by endophytic entomopathogenic fungi** Aragón, Sandra[1,2], Cotes, Alba Marina[2], Vidal, Stefan[1], [1]Georg-August-Universität Göttingen, Department of Crop Sciences, Göttingen, Germany. [2]Biothechnology and Bioindustry Center, Colombian Corporation for Agricultural Research Corpoica, Mosquera, Colombia

12:30–14:00 **LUNCH** Mensa

Contributed Papers Wednesday, 13:15-14:00. **P203**

JIP EDITORIAL BOARD

Student Workshop Wednesday, 12:30-14:00. **P2**

HOW TO WRITE A PAPER

Moderators: Rich Humber, Mark Goettel and Yukino Inoue

Contributed Papers Wednesday, 14:00-16:00. **P4**

MICROSPORIDIA 1

Moderator: Susan Bjørnson

14:00 **169 Effects of the microsporidium *Nosema adaliae* on the multicoloured Asian lady beetle, *Harmonia axyridis*** Bryan Ellis, Susan Bjørnson, Department of Biology, Saint Mary's University, Halifax, Nova Scotia, Canada

14:15 **170-STU Effects of two microsporidia from lady beetles on the green lacewing, *Chrysoperla carnea*** Jackline Sirisio, Susan Bjornson, Department of Biology, Saint Mary's University, Halifax, Nova Scotia, Canada

14:30 **171 Features of the genomes of microsporidia in mosquitoes: status and preliminary findings** James J. Becnel[1], Christopher Desjardins[2], Neil Sanscrainte[1], and Christina Cuomo[2], [1]Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL, USA, [2]Genome Sequencing Center for Infectious Disease, Broad Institute of MIT and Harvard, Cambridge, MA, USA

14:45 **172 Multi-gene phylogeny applied to the taxonomy of microsporidian parasites of crustacean hosts** K.S. Bateman[1], R. Kerr[1], D. Wiredu- Boakye[2], B. Williams[2], G.D. Stentiford[1], [1]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset, UK, [2]Biosciences, University of Exeter, Devon, UK

15:00 **173-STU Understanding the evolutionary loss of glycolysis in intranuclear crab microsporidians** Dominic Wiredu Boakye[1], Bryony Williams[1]; Grant Stentiford[2], and Thomas Williams[3], [1]College of Life and Environmental Sciences. University of Exeter, Exeter; UK, [2]Centre of Environment, Fisheries and Aquaculture Science, CEFAS, Weymouth, UK; [3]Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne, Tyne and Wear, UK

15:15 **174-STU Temporal trends and the effect of seasonal temperature on the prevalence of *Nosema* spp. in *Apis mellifera* in north-east Germany** Anto Raja Dominic[1,3], Sebastian Gisder[2], Elke Genersch[2], Andreas Linde[1], [1]Hochschule für nachhaltige Entwicklung Eberswalde, Dept. of Forest and Environment, Eberswalde, Germany, [2]Länderinstitut für Bienenkunde Hohen Neuendorf e.V., Hohen Neuendorf, Germany, [3]Freie University, Berlin, Germany

15:30 **175 STU Characterising putative virulence factors of the bee pathogen *Nosema ceranae*** Graham Thomas, Ken Haynes; University of Exeter, UK

15:45 **176 Detection of Microsporidia in Gammarids in the Delta of the Kuban River (Azov Sea, Russia)** Yuri Tokarev[1], Vladimir Voronin[2], Egor Rusakovich[3], Irma Issi[1], [1]All-Russian Institute of Plant Protection, St. Petersburg, Russia; [2]St. Petersburg Veterinary Medical Academy, St. Petersburg, Russia; [3]Herzen State Pedagogical University of Russia, St. Petersburg, Russia

Contributed Papers Wednesday, 14:15-15:45. **P3**

MICROBIAL CONTROL 3

Moderator: Stefan Jaronski

14:15 **178-STU Synthesis and Characterization of fungus mediated silver nanoparticle for the toxicity on filarial Vector, *Culex quinquefasciatus*** Siva Kamalakannan[1], Chandrakasan Gobinath[2], Sivapunyam Ananth[3], Kadarkarai Murugan[1]; [1]Division of Entomology, Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu,India; [2]Bio control laboratory, Department of Biotechnology and Genetic Engineering, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India; [3]Insect control division, Department of Biotechnology, Annai Arts and Science College, Kumbakonam, Tamil Nadu, India

14:30 **179-STU Entomopathogenic fungi as endophytes: interaction with phytohormones** Dalia Muftah Alkhayat; Katharina Döll, Petr Karlovsky, Stefan Vidal; Institute for Plant Protection and Plant Pathology, Georg-August University, Göttingen, Germany

14:45 **180 Pathogenicity of three entomopathogenic fungi on larvae and adults of the sisal weevil: The less the better?** Vasiliki Gkounti[1], Markogiannaki Dimitra[2], Dimitris Kontodimas[2], [1]SLU, Sweden, [2]Benaki Phytopathological Institute, Greece

15:00 **181 Understanding *Beauveria bassiana* infection within its host *Triatoma infestans*: time course expression of genes encoding fungal toxic nonribosomal peptides and insect humoral immune proteins** Luciana S. Lobo[1,2], Éverton K. K. Fernandes[2], Christian Luz[2], M. Patricia Juárez[1], Nicolás Pedrini[1], [1]Instituto de Investigaciones Bioquímicas de La Plata (CCT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, La Plata, Argentina; [2]Instituto de Patologia Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás, Goiânia, Brazil

15:15 **182 Compatibility of herbicides used in olive orchards with a *Metarhizium brunneum* strain used for the control of the olive fly preimaginals in the soil** Enrique Quesada-Moraga, Inmaculada Garrido-Jurado, Meelad Yousef, University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain

15:30 **183 The Seed Corn Maggot and *Metarhizium* are Related to Maize Yield in an Organic, Cover Crop-Based Farming Systems Experiment** Mary Barbercheck; Christina Mullen, Department of Entomology, Penn State University, University Park, USA

Contributed Papers Wednesday, 14:00-16:00. **P1**

VIRUSES 5

Moderators: Bryony Bronning and Alicia Timm

14:00 **184 Soybean aphid viruses exploit contrasting transmission strategies** Diveena Vijayendran, Sijun Liu, Bryony C. Bonning, Department of Entomology, Iowa State University, Ames, USA

14:15 **185 Characterization of mechanisms involved in the transmission of a lepidopteran densovirus** Cécilia Multeau[1], Doriane Mutuel [2], Manuela Rakotomanga[2], Anne Kenaghan[2], Clément Bousquet[2], Rémy Froissart[3-4], Nathalie Volkoff[2] and Mylène Ogliastro[2] ; [1]InVivo AgroSolutions, Valbonne, France; [2]INRA, UMR 1333 DGIMI, INRA, Montpellier, France; [3]CNRS, UMR 5290 MIVEGEC, Montpellier, France; [4]CIRAD-SupAgro, UMR 385 BGPI, Montpellier, France

14:30 **186 Discovery of circular single-stranded DNA viruses in top insect predators** Karyna Rosario[1], Anisha Dayaram[2], Jessica Ware[3], Milen Marinov[2], Mya Breitbart[1], Arvind Varsani[2]; [1]College of Marine Science, University of South Florida, Florida, USA; [2]School of Biological Sciences, University of Canterbury, Christchurch, New Zealand; [3]School of Environmental and Biological Sciences, Rutgers University, New Jersey, USA

14:45 **187-STU Single-stranded DNA viruses in marine crustaceans** Ryan Schenck[1]; Karyna Rosario[1]; Rachel Harbeitner[1]; John Cannon[2]; Mya Breitbart[1], [1]University of South Florida College of Marine Science, Tampa, Florida, USA; [2]University of South Florida College of Medicine Department of Pediatrics, USA

15:00 **188 Remarkable diversity of endogenous viruses in the genome of an isopod crustacean** Julien Thézé, Sébastien Leclercq, Bouziane Moumen, Richard Cordaux, Clément Gilbert; Université de Poitiers, Laboratoire Ecologie et Biologie des Interactions - UMR CNRS 7267, Equipe Ecologie Evolution Symbiose, Poitiers Cedex, France

15:15 **189 Iteraviruses (Densovirinae) from monarch and black swallowtail butterflies and slug caterpillar moths and characterization of their expression strategies** Qian Yu, Max Bergoin, and Peter Tijssen, INRS-Institut Armand-Frappier, Laval, QC, Canada

15:30 **190 Remarkable genetic diversity of single-stranded DNA viruses in cultured shrimps and crickets** Hanh T. Pham, Qian Yu, Max Bergoin, Peter Tijssen, INRS-Institut Armand-Frappier, Université du Québec, Laval, QC, Canada

15:45 **191 How do vine mealybug, grapevine leafroll-associated virus and grapevine interact on a molecular level?** Alicia Eva Timm[1] & Annette Reineke[2], [1]Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa, [2]Institut für Phytomedizin, Geisenheim Hochschule, Geisenheim, Germany

Contributed Papers Wednesday, 14:00-15:45 **P5**

BACTERIA 4

Moderators: Yulin Gao and Neil Crickmore

14:00 **192 Analysis of the bacterial community of the insect pest *Lymantria dispar* during its life cycle** Zane Metla[1,2,3]; Monika Maurhofer[2], Liga Jankevica[1,3], [1]Plant Pathology, Institute of Integrative Biology (IBZ). Swiss Federal Institute of Technology, Switzerland; [2]Laboratory of Experimental Entomology, Institute of Biology, Univ. Latvia, Latvia; [3]Univ.of Daugavpils, Latvia

14:15 **193 Contacting microbe induce grooming behaviour in Drosophila** Aya Yanagawa[1,2], Tsuyoshi Yoshimura[1], Hata Toshimitsu[1] and Frédéric Marion-Poll[2,3], [1]Kyoto University, Uji, Japan; [2]CNRS, Laboratoire Evolution, Génomes et Spéciation, Gif-sur-Yvette, France; [3]AgroParisTech, Département Sciences de la Vie et Santé, Paros, France

14:30 **194 Cultivable gut bacteria of scarabs inhibit *B. thuringiensis* multiplication** Yueming Shan[1,2], Changlong Shu[2], Neil Crickmore[3], Chunqin Liu[4], Wensheng Xiang[1], Fuping Song[2], Jie Zhang[2], [1]School of Life Science, Northeast Agricultural University, Harbin, P.R. China; [2]State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China; [3]School of Life Sciences, University of Sussex, Falmer, Brighton, UK; [4]Cangzhou Academy of Agricultural and Forestry Sciences, Cangzhou, P.R. China

14:45 **195 Interactions between the Med fly *Ceratitis capitata* (Wied.) and a new *Bacillus cereus sensu lato* strain** Luca Ruiu[1,2], Giovanni Falchi[2], Ignazio Floris[1], Maria G. Marche[1,2], Maria E. Mura[2], Alberto Satta[1], [1]Dipartimento di Agraria, University of Sassari, Italy, [2]Bioecopest Srl. Technology Park of Sardinia, Italy

15:00 **196 Long-term effect of *Bacillus thuringiensis* subsp. *israelensis* application on *B. cereus* group populations in Swedish riparian wetland soils** Salome Schneider[1], Tania Tajrin[1], Niels B. Hendriksen[2], Jan O. Lundström[3], Petter Melin[1], Ingvar Sundh[1], [1]Department of Microbiology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, [2]Department of Environmental Science, Aarhus University, Roskilde, Denmark, [3] Mosquito and Environment Group, Program for Population and Conservation Biology, Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden

15:15 **197 Proteomics of *Brevibacillus laterosporus* and its insecticidal action against noxious Diptera** Maria G. Marche[1,2], Maria E. Mura[1], Giovanni Falchi[1], Luca Ruiu[1,2], [1]Dipartimento di Agraria, University of Sassari, Italy, [2]Bioecopest Srl. Technology Park of Sardinia, Italy

15:30 **198-STU Outer membrane vesicles are vehicles for the delivery of *Vibrio* virulence factors to oyster immune cells** Audrey S. Vanhove[1], Marylise Duperthuy[1,2], Guillaume M. Charrière[1], Frédérique Le Roux[3], David Goudenège[3], Benjamin Gourbal[4], Sylvie Kieffer-Jaquinod[5], Yohann Couté[5], Sun N. Wai[2] and Delphine Destoumieux-Garzón[1], [1]Ecology of coastal marine systems, CNRS, Ifremer, IRD, University of Montpellier, France; [2]Umea University, Department of Molecular Biology, The Laboratory for Molecular Infection Medicine Sweden (MIMS), Sweden; [3]Integrative Biology of Marine Models, CNRS, Ifremer, Université Pierre et Marie Curie. Station Biologique de Roscoff, France; [4]Université de Perpignan, Ecology and Evolution of Interactions, France; [5]Université Grenoble-Alpes, CEA, iRTSV, Biologie à Grande Echelle; INSERM, France

16:00–16:30 **BREAK**

Wednesday, 16:30-18:30. **Philosophicum**

POSTERS

Posters should be displayed from Monday UNTIL NOT LATER THAN 18:00 THURSDAY

BACTERIA

BA-1 A New Local Bio-Insecticide: Developing, Optimization, Toxicity and Determination of Activity Kazım Sezen, Remziye Nalcacioglu, Ismail Demir, Hüseyin Tepe, Islam Yildiz, Ardahan Eski, Zihni Demirbag, Karadeniz Technical University, Faculty of Science, Department of Biology, Trabzon, Turkey

BA-2 `*Candidatus* Rickettsiella isopodorum´, a new lineage of intracellular bacteria infecting woodlice Regina G. Kleespies[1]; Andreas Leclerque[1,2]; [1]Institute for Biological Control, Julius Kühn Institute (JKI), Germany, [2]Geisenheim University, Institute for Microbiology and Biochemistry, Geisenheim, Germany

BA-3-STU Analysis and characterization of binary AB toxins in the honey bee pathogen *Paenibacillus larvae* Julia Ebeling, Lena Poppinga, Anne Fünfhaus, Elke GenerschInstitute for Bee Research, Hohen Neuendorf, Brandenburg, Germany

BA-4 Interplay of Regulators Controlling Fit Insect Toxin Expression in the Biocontrol Bacterium *Pseudomonas protegens* Nicola Imperiali[1], Flavia Büchler[1], Maria Péchy-Tarr[1], Peter Kupferschmied[1], Monika Maurhofer[2], and Christoph Keel[1]; [1]Department of Fundamental Microbiology, University of Lausanne, Switzerland, [2]Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland

BA-5-STU Identification and Characterization of *Bacillus thuringiensis* Strains with Nematicidal Activity Luis A. Verduzco-Rosas and Jorge E. Ibarra. CINVESTAV IPN, Irapuato, Mexico

BA-6 Evaluation of Culture media for maximal growth, Cry toxin production and insecticidal toxicity of *Bacillus thuringiensis* M. Tripathi [1], A. Kumari[2], L. Saravanan[3] G.T. Gujar[4], [1,4]Division of Entomology, Indian Agricultural Research Institute, New Delhi, [2]TERI, India Habitat Centre, New Delhi, [3]Directorate of Medicinal and AromaticPlants Research, Anand

BA-7 Gene organization of large plasmids of novel mosquitocidal *Bacillus thuringiensis* TK-E6 Mayu Noda, Naruhei Okamoto, Kimie Hayasaki, Yoshinao Azuma, and So Takebe; Faculty of Biology-Oriented Science and Technology, Kinki University, Wakayama , Japan

BA-8-STU Testing of Vip3 proteins for the control of caterpillar pests Iñigo Ruiz de Escudero [1,2], Núria Banyuls [3], Yolanda Bel [3], Mireya Maeztu [1], Baltasar Escriche [3], Delia Muñoz [2], Primitivo Caballero [1,2], Juan Ferré [3], [1]Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadía, Mutilva, Navarra, Spain. [2]Laboratorio de Entomología Agrícola y Patología de Insectos, Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain. [3]Departamento de Genética, Facultad de CC. Biológicas, Universitat de València, Valencia, Spain

BA-9 Interactions between Cry and Vip proteins from *Bacillus thuringiensis* against different lepidopteran pests Ana Rita Nunes Lemes[1], Camila Chiaradia Davolos[1], Paula Cristina Brunini Crialesi Legori[1], Odair Aparecido Fernandes[2], Juan Ferré[3], Manoel Victor Franco Lemos[1], Janete Apparecida Desiderio[1]; [1]Dpto de Biologia Aplicada à Agropecuária, UNESP/Campus de Jaboticabal, Brazil, [2]Dpto de Fitossanidade, UNESP/Campus de Jaboticabal, Brazil, [3]Dpto de Genética, Universidade de València, Spain

BA-10 Cry1Ac and Cry1F toxicity and binding sites study in two important soybean pests, *Anticarsia gemmatalis* and *Chrysodeixis (=Pseudoplusia) includens.* Yolanda Bel 1, Ken Narva 2, Joel Sheets 2, Baltasar Escriche [1], [1] Dept. Genetics, ERI BioTecMed, Universitat de València, Dr. Moliner, Burjassot, Valencia, SPAIN; [2] Dept. Biochemistry/Mol. Biology. Dow AgroSciences, Zionsville Rd. Indianapolis, USA

BA-11-STU *In vivo* and *in vitro* binding of Vip3Aa to *Spodoptera frugiperda* midgut and characterization of binding sites using ^{125}I-radiolabeling Maissa Chakroun and Juan Ferré, Department of Genetics, University of Valencia, 46100-Burjassot (Valencia), Spain

BA-12 Comparative histopathology of two novel bacterial insecticidal proteins in *Tenebrio molitor* and *Diabrotica virgifera virgifera* larvae Heba Abdelgaffar[1]; Cris Oppert[2], Jayme Williams[2], Deepa Balasubramanian[2], Juan Luis Jurat-Fuentes[1]; [1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville (TN), USA; [2]Bayer CropScience, Morrisville (NC), USA

BA-13-STU Role of ABC-C2 in the interactions of *Heliothis virescens* with its host plants and Bt toxins Anne Karpinski, Yannick Pauchet, Heiko Vogel and David Heckel, Department of Entomology, Max Planck Institute for Chemical Ecology, Jena Germany

BA-14-STU **AminomemtidaseN in *Popillia japonica* Newman larvae is putative *Bacillus thuringiensis* Cry8Da toxin receptor** Yuu Taniguchi, Takuya Yamaguchi, Hisanori Bando, Shin-ichiro Asano, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

BA-15 **A Whole Genome Approach to Determine Cadherins associated with Bt toxicity in the Diamondback Moth, *Plutella xylostella*** Youngjin Park and Yonggyun Kim, Department of Bioresource Sciences, Andong National University, Andong, South Korea

BA-16 **RNA Interference of Integrin subunit β1 Impairs Development and Immune Responses of the Oriental tobacco budworm, *Helicoverpa assulta* against Bacteria** Youngjin Park and Yonggyun Kim, Department of Bioresource Sciences, Andong National University, Andong, South Korea

BA-17 **A natural hybrid of a *B. thuringiensis* Cry2A toxin implicates domain I in specificity determination.** Guihua Chen[1,3], Changlong Shu[1], Jacob Evans[2], Fuping Song[1], Guoxun Li [3], Neil Crickmore [2], Jie Zhang[1]; [1]State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P. R. China; [2]School of Life Sciences, University of Sussex, Falmer, Brighton, UK; [3] College of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao, China

BA-18 ***Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of *Crioceris quatuordecimpunctata* to *Beauveria bassiana* infection** Yulin Gao[1], Zhongren Lei[1], Xuenong Xu[1], [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193,PR. China

BA-19 **InterVening Sequence (IVS) elements as genetic markers for the differential diagnosis of arthropod-associated *Rickettsiella* bacteria** Christina Schuster [1]; Katharina Saar[1]; Regina G. Kleespies[1]; Andreas Leclerque[1,2], [1]Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; [2]Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany

BA-20 **Type IV Secretion System (T4SS) substrates as potential virulence factors of arthropod-pathogenic *Rickettsiella* bacteria** Andreas Leclerque, Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany

BA-21 **Unbalanced Polyphosphate Levels Impair Insect Pathogenicity in Plant-Beneficial *Pseudomonas protegens*** Maria Péchy-Tarr[1], Nicolas Wenner[1], Peter Kupferschmied[1], Romane Keller[1], Monika Maurhofer [2], Christoph Keel[1]; [1]Department of Fundamental Microbiology, University of Lausanne, Switzerland; [2]Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland

BA-22-STU ***Paenibacillus larvae* and the virulence factor SplA- an ERIC II specific S-layer Protein** Henriette Knispel, Lena Poppinga, Anne Fünfhaus, Elke Genersch*, Institute for Bee Research, Hohen Neuendorf; Division of Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany

BA-23 **Influence of (varying) population size on host-parasite coevolution: an experimental approach** Andrei Papkou, Rebecca Schalkowski, Mike-Christoph Barg, Ines Braker, Hinrich Schulenburg, Evolutionary Ecology Genetics, Zoological Institute, CAU Kiel. Address for Correspondence: apapkou@zoologie.uni.kiel.de

BA-24 **An *in vivo* experimental evolution system for analyzing bacterial adaptation and evolution of *Bacillus cereus sensu lato* in an insect model** Rafael Patiño Navarrete[1,2], Isabelle Jéhanno[1,2], Christina Nielsen-Leroux[1,2] and Vincent Sanchis[1,2], [1]INRA, UMR1319 Micalis, F-78350 Jouy-en-Josas, France, [2]AgroParisTech, UMR Micalis, F-78350 Jouy-en-Josas, France

DISEASES OF BENEFICIAL INVERTEBRATES

DB-1-STU **Identification and Characterization of Immune Inhibitor A Metalloprotease of the Honey Bee Pathogen *Paenibacillus larvae*** Birte Arlt[1,2]; Gillian Hertlein[1]; Lena Poppinga[1]; Eva Garcia-Gonzalez[1]; Elke Genersch[1,3], [1]Institute for Bee Research Hohen Neuendorf, Hohen Neuendorf, Germany; [2]Technische Universität Berlin, Institute of Biotechnology, Berlin, Germany; [3]Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany

DB-2 **Awareness and Concept of Insects in a Korean Population** Sung Min Bae, Tae Young Shin, Jae Bang Choi, Won Seok Kwak, Yong Oh Ahn, See Nae Lee, In Hui Kim, Ra Mi Woo, Dong Jun Kim and Soo Dong Woo, Department of Agricultural Biology, Chungbuk National University, Chungju, Korea

DB-3 **Virus Epizootiology in Managed and Native Bee Populations** John P. Burand[1]; Matthew Boucher[2]; Anne Averill[3], Departments of [1]Microbiology, [2]Biology and [3]Environmental Conservation, University of Massachusetts - Amherst, Amherst, USA

DB-4 **Honeybee Virus Epizootiology in Bee Populations in Connecticut, USA** John P. Burand[1]; Shuning Zheng[2]; Kimberly Stoner[3], [1]Department of Microbiology, [2]Graduate Program in Molecular and Cellular Biology, University of Massachusetts - Amherst, Amherst, USA and [3]Connecticut Agricultural Experiment Station, New Haven, USA

DB-5 **High-throughput sequence analysis of the change in expression profile of Ig2-, Ig3- and Ig7- variant domains in *Carcinus maenas* Down Syndrome Cell Adhesion (*CmDscam*) mRNAs in response to pathogenic infection** Chris Hauton[1]; John A. Hammond[2], [1]School of Ocean and Earth Sciences, University of Southampton, National Oceanography Centre, Southampton, Hants, UK; [2]Immunogenetics Group, The Pirbright Institute, Pirbright, Woking, UK

DB-6 **A novel pathogenic *Paenibacillus* strain of *Biomphalaria glabrata,* an intermediate host for schistosomiasis** David Duval[1,2], Richard Galinier[1,2], Gabriel Mouahid[1,2], Eve Toulza[1,2], Anne Rognon[1,2], Nathalie Arancibia[1,2],Jean Francois Allienne[1,2], Guillaume Mitta[1,2], André Théron[1,2], Benjamin Gourbal[1,2], [1]CNRS, UMR 5244, Ecologie et Evolution des Interactions (2EI), Perpignan, France, [2]Université de Perpignan Via Domitia, Perpignan, France

DB-7 **Venom from the ectoparasitic wasp *Habrobracon hebetor* activates calcium-dependent processes of haemocytic degradation in *Galleria mellonella* larvae** Natalia. A. Kryukova[1], Ekaterina A.Chertkova[1], Alexandra D. Semenova[2], Yuri I.Glazachev[2], Irina A. Slepneva[2], Victor V. Glupov[1], [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

DB-8 **Histopathological analyses of different tissues of diseased honey bees (*Apis mellifera*)** Lena Poppinga[1], Heike Aupperle[2], Elke Genersch[1], [1]Institute for Bee Research, Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany; [2]Laboklin GmbH & Co KG, Bad Kissingen, Germany

DB-9 **New findings in genome of *Apis mellifera* filamentous virus** Lukasz Rabalski[1], Urszula Grzeda[2]; Grazyna Topolska[2]; Martyna Krejmer[1]; Boguslaw Szewczyk[1], [1]Department of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology of the University of Gdansk and Medical University of Gdansk, Gdansk, Poland; [2]Laboratory of Bee Diseases, Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

DB-10 **Development of prototypes of rapid molecular diagnostic tests for pathogens of honeybees (*Apis mellifera* L.) on chromatographic NALF platform (Nucleic Acid Lateral Flow)** Adriano Ragni[1]; Francesca Tabarrini[1]; Mario Carucci[1]; Claudio E. Lorenzetti[1]; Antonella Cersini[2]; Silvia Puccica[2]; Valeria Antognetti[2]; Marcella Milito[2]; Alessandra Giacomelli[2]; Giovanni Formato[2]; Francesco Panara[3], [1]RAPID BIOTECH, Perugia; [2]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma; [3]ENEA - Centro Ricerche Trisaia S.S. 106 Ionica, Rotondella Matera, Italy

DB-11 **What Kind of Insects Do You Like?** Tae Young Shin, Sung Min Bae, Jae Bang Choi, Won Seok Kwak, Yong Oh Ahn, See Nae Lee, In Hui Kim, Ra Mi Woo, Dong Jun Kim and Soo Dong Woo, Department of Agricultural Biology, Chungbuk National University, Chungju, Korea

DB-12 **A muscle-infecting microsporidium infecting pink shrimp (*Pandalus montagui*) from Europe: closing in on the type species of *Thelohania*?** Stentiford, G.D., Ross, S., Kerr, R., Bateman, K.S., European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset DT4 8UB, UK

FUNGI

FU-1-STU **Monitoring of entomopathogenic fungi in *Metarhizium* and *Beauveria* treated fields** Emese Balog, Do Van Hung, Zoltán Mayer, György TurócziSzent István University, Plant Protection Institute, Gödöllő, Hungary

FU-2 **Distribution of insect-pathogenic soil fungi in agricultural and forest ecosystems in Georgia** Medea Burjanadze[1], Richard Humber[2], Mariam Arjevanidze[1], Tea Abramishvili[1], Giuli Tsereteli[1], Manana Lortkipanidze[3], [1]Agricultural University of Georgia, Department of Forest protection, Georgia; [2]USDA-ARS BioIPM Resarch, RW Holley Center for Agriculture and Health, Ithaca, NY., USA; [3]Ilis State, University Institute of Zoology, Georgia.

FU-3 **Diversity of Entomopathogenic fungi in different citrus cropping systems in Brazil** Celeste P. D'Alessandro, Vanessa da Silveira Duarte, Elisa S. Dominguez, Ana C. Oliveira dos Santos, Italo Delalibera Jr. Department of Entomology and Acarology, ESALQ, University of São Paulo, Av. Pádua Dias 11, CP. 9, Piracicaba, São Paulo, Brazil.

FU-4 **The Entomopathogenic Fungus *Isaria* for Pest Insect Control in Vegetables** Katharina Saar[1]; Andreas Leclerque[2]; Dietrich Stephan[1], [1]Institute for Biological Control, Julius Kühn-Institut (JKI), Darmstadt, Germany; [2] Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany

FU-5 **Prevalence of *Beauveria pseudobassiana* among tick-associated fungal isolates from the Republic of Moldova** Natalia V. Munteanu[1]; Polina V. Mitkovets[2]; Galina V. Mitina[2]; Alexandru Movila[1]; Yuri S. Tokarev[2]; Andreas Leclerque[3,4], [1]Institute of Zoology, Academy of Sciences of Moldova, Chisinau, Republic of Moldova; [2]All-Russian Institute for Plant Protection, Saint-Petersburg, Russia; [3]Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; [4]Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany.

FU-6 **Diversity and abundance of entomopathogenic fungi on strawberry crops in Brazil** Thiago Rodrigues de Castro[1,2]; Lívia Maria Alves Porto[1], Jørgen Eilenberg[2], Italo Delalibera Júnior[1], [1]University of São Paulo (ESALQ), Brazil; [2]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark.

FU-7 **Abundance and diversity of *Metarhizium* spp. in an agricultural landscape in Sweden** Salome Schneider[1], Stefan Stranne[1], Hanna Friberg[2], Ingvar Sundh[1]; [1]Department of Microbiology and [2]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden.

FU-8 **Diversity and distribution of entomopathogenic fungi in Czech Republic soils** Kateřina Šimáčková[1,2]; Jana Kročáková[2]; Andrea Bohatá[2]; Noemi Herrero[1]; [1]Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i. Institute of Entomology, České Budějovice, Czech Republic; [2]University of South Bohemia, Faculty of Agriculture, České Budějovice, Czech Republic

FU-9 **Entomopathogenic fungi as plant growth enhancers** Surendra K. Dara[1], Sumanth S. Dara[2], Suchitra S. Dara[3]; [1]Division of Agriculture and Natural Resources, University of California; [2]Stockdale High School, Bakersfield, USA; [3]Warren Junior High School, Bakersfield, USA.

FU-10 **The entomopathogenic fungus *Beauveria bassiana* improves the growth of *Triticum aestivum* and *Triticum durum*** Antonio Rafael Sánchez-Rodríguez[1], María del Carmen del Campillo[2], Inmaculada Garrido-Jurado[1], Enrique Quesada-Moraga[1]; [1]Departamento de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, España, [2]Departamento de Agronomía, Universidad de Córdoba, España

FU-11-STU **Interactions between cowpea plants vs. *Metarhizium* spp. entomopathogenic fungi** Patrícia S. Golo[1]; Walquíria Arruda[2]; Flávia R. S. Paixão[2]; Fabrício

M. Alves[2]; Éverton K. K. Fernandes[2]; Donald W. Roberts[3]; Vânia R. E. P. Bittencourt[1]; [1]Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil; [2]Universidade Federal de Goiás, Goiânia, Brazil; [3]Utah State University, Logan, USA.

FU-12 Biological control in oilseed rape: An attempt to establish the entomopathogenic fungus *Beauveria bassiana* as an endophyte in oilseed rape plants Cornelia Ullrich[1]; Saoussene Talbi[1]; Andreas Leclerque[1,2]; Frank Rabenstein[3]; Regina G. Kleespies[1]; [1]Institute for Biological Control, Julius Kühn Institute (JKI), Germany,[2]Hochschule Geisenheim, University, Geisenheim, Germany; [3]Julius Kühn Institute, Quedlinburg, Germany

FU-13 Azygo- and zygospore formation of *Neozygites floridana* in the two-spotted spider mite (*Tetranychus urticae*) in strains from tropical and temperate regions Karin Westrum[1]; Vanessa S. Duarte[2]; Richard A. Humber[3]; Italo Delalibera Jr[2]; Ingeborg Klingen[1]; [1]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Ås, Norway; [2]ESALQ – University of São Paulo, Piracicaba, Brazil; [3]USDA-ARS BioIPM Research, Ithaca, NY, USA.

FU-14 Susceptibility of *Biomphalaria glabrata* egg masses to fungal infection Glennyha F. Duarte, Juscelino Rodrigues, Éverton K. K. Fernandes, Christian Luz Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil

FU-15 Antimicrobial, Antioxidant and Anticancer Activity of Culture Filtrates from Entomopathogenic Fungi Tae Young Shin, Sung Min Bae, Jae Bang Choi, Won Seok Kwak, Yong Oh Ahn, See Nae Lee, In Hui Kim, Ra Mi Woo, Dong Jun Kim and Soo Dong Woo; Department of Agricultural Biology, Chungbuk National University, Chungju, Korea

FU-16 Evolutionary-ecological strategies of *Metarhizium robertsii* Olga Yaroslavtseva, Vadim Kryukov, Ivan Dubovskiy, Maxim Tyurin,Victor Glupov; Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

FU-17 Mycelial and conidial thermotolerance **of *Metarhizium anisopliae* s.l. IP 46 and *Metarhizium robertsii* ARSEF 2575** Flávia R. S. Paixão[1]; Elen R. Muniz[1]; Cíntia C. Bernardo[1]; Gabriel M. Mascarin[2]; Christian Luz[1]; Éverton K. K. Fernandes[1]; [1]Universidade Federal de Goiás, Goiânia, Brazil; [2]Embrapa Arroz e Feijão, Goiânia, Brazil.

FU-18 Delayed germination of heat-stressed conidia of *Metarhizium anisopliae* on tick cuticle Lucas P. Barreto[1]; Fabrício M. Alves[1]; Christian Luz[1]; Gabriel M. Mascarin[2]; Donald Roberts[3]; Walquíria Arruda[1]; Éverton K. K. Fernandes[1]; [1]Universidade Federal de Goiás, Goiânia, Brazil; [2]Embrapa Arroz e Feijão, Goiânia, Brazil; [3]Utah State University, Logan, USA.

FU-19 Influence of environmental factors on insects resistance to anamorphic fungi Vadim Kryukov; Ivan Dubovskiy, Olga Yaroslavtseva, Maxim Tyurin, Natalia Kryukova, Victor Glupov; Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

FU-20 Intraspecific and interspecific variation in osmotolerance of entomopathogenic fungi Claudineia A. S. Araujo[1], Clara C. Oliveira[1], Marília A. Rodrigues[1], Breno Pupin[1], Luciana P. Dias[1], John E. Hallsworth[2], and Drauzio E. N. Rangel[1]. [1]Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, Brazil. [2]School of Biological Sciences, MBC, Queen's University Belfast, UK

FU-21 Different intensities of visible light during mycelial growth induce differently the conidial tolerance to menadione in *Metarhizium robertsii* Luciana P. Dias[1,2], Drauzio E. N. Rangel[1], [1]Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, Brazil.

FU-22 Effect of *Metarhizium* spp. growth media on the accumulation of destruxins in a 10-L stirred tank reactor Martin Parth[1], Judith Taibon[1,2], Hermann Strasser [1]; [1]Institute of Microbiology, Leopold-Franzens University Innsbruck, Austria; [2]Institute of Pharmacy / Pharmacognosy, Leopold-Franzens University Innsbruck, Austria

FU-23 Evaluation of destruxin A production in four strains of *Metarhizium* by capillary electrophoresis Alex Ríos-Moreno[1], Azahara Carpio[2], Inmaculada Garrido-Jurado[1], Lourdes Arce[2], Miguel Valcárcel[2], Enrique Quesada-Moraga[1]; [1]Department of Agricultural and Forestry Sciences, ETSIAM, University of Cordoba. Campus de Rabanales. Edificio C4 Celestino Mutis. Cordoba, Spain, [2]Department of Analytical Chemistry, University of Cordoba, Annex C3 Building, Nanochemistry and Fine Chemistry Research Institute (IUIQFN), Campus of Rabanales, Cordoba, Spain

FU-24 Entomopathogenic fungal genera and the 1F=1N standard: The shape of the future begins to emerge Ryan M. Kepler[1], Stephen A. Rehne [1], Richard A. Humbe [2], [1]USDA-ARS Systematic Mycology and Microbiology Laboratory, Beltsville, Maryland, USA; [2]USDA-ARS Biological IPM Research, RW Holley Center,Ithaca, New York, USA

FU-25 Genotyping of Georgian isolates of entomopathogenic fungi *Beauveria* spp. Nana Kunelauri[1], Vladimer Baramidze[1], Medea Burjanadze[1], Ekaterine shubladze[1], Eka Mikeladze[1], [1]Agricultural University of Georgia, Tbilisi, Georgia, G. Tevzadze Laboratory of Microbial Genomics, [2]Agricultural University of Georgia, Deapartment of Forest Protection, Tbilisi, Georgia

FU-26 Genetic characterization, fungicide sensitivity, and aphicidal potential of *Lecanicillium* fungi from Argentina Romina Manfrino[1,2]; Christina Schuster[3]; Julieta Tornesello Galván[1]; Katharina Saar[3]; Juan J. García[1]; Claudia C. López Lastra[1]; Andreas Leclerque[3,4], [1]Centro de Estudios Parasitológicos y de Vectores (CEPAVE), La Plata (BsAs), Argentina; [2]Instituto Nacional de Tecnología Agropecuaria (INTA), Rafaela (Santa Fe), Argentina; [3]Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; [4]Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany

FU-27 Species-specific PCR assay to identify and discriminate *M. pingshaense*, *M. anisopliae*, *M. brunneum,* and *M. robertsii* Johanna Mayerhofer[1], Andy Lutz[1], Franco Widmer[1], Stephen A. Rehner[2], Ryan M. Kepler[2], Adrian Leuchtmann[3], Jürg Enkerli , [1]Molecular Ecology, Institute for Sustainability Sciences, Agroscope, Reckenholzstrasse Zurich, Switzerland; [2]Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland, USA; [3]Plant Ecological Genetics, Institute of Integrative Biology, ETH Zurich, Switzerland

FU-28 **Species identification of entomopathogenic fungi of the genus *Lecanicillium* (=*Verticillium lecanii* s.l.) by mitochondrial gene sequences** Galina V. Mitina, Yuri S. Tokarev, Igor A. Kazartsev, All-Russian Institute for Plant Protection, Saint-Petersburg, Russia

FU-29 **The genomic basis for evolved resistance to *Beauveria bassiana* in *Drosophila melanogaster*** Parvin Shahrestani[1], John Vandenberg[2], Michael Griggs[2], Stephen Wraight[2], Yonathan Estrella[1], Susan Rottschaefer[1], Andrew Clark[3], Brian Lazzaro[1], [1]Department of Entomology, Cornell University, Ithaca NY, USA; [2]USDA Agricultural Research Service, Ithaca NY, USA; [3]Department of Molecular Biology and Genetics, Cornell University, Ithaca NY, USA

FU-30-STU **Behavioral control of malarial mosquito by entomopathogenic fungi: Death as the vector** Minehiro Ishii [1]; Masanori Koike [2]; Daigo Aiuchi [2], [1]The United Graduate School of Agricultural Sciences, Iwate University, Japan; [2] Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Japan.

FU-31 **Effect of *Metarhizium brunneum* strain LRC112 and *M. anisopliae* F52 on non-target Carabid Beetles** Alida F. Janmaat [1], Chera Rempel [1], Rita Quik [1], Todd Kabaluk [2], Manon Peyre [2], Remi Thomasset [2], [1] Biology Department, University of the Fraser Valley, Abbotsford, BC, Canada; [2] Agriculture and Agri-Food Canada. Agassiz, BC, Canada

FU-32 **Effect of a local strain of the fungus against *Corythucha ciliata* (Say) and *Glyphodes pyloalis* (Walker) in Georgia** Manana Kereselidze, Mzia Beruashvili, Mzagho Lobzhanidze, Agricultural University of Georgia, Tbilisi, Georgia

FU-33 **The effect of pesticides used in strawberry and soybean on the mite pathogenic fungus *Neozygites floridana*** Thiago Rodrigues de Castro[1]; Samuel Roggia[1,4], Vitalis Wafula Wekesa[2]; Ingeborg Klingen[3]; 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, [4]The Brazilian Agricultural Research Corporation – Embrapa Soybean, Brazil.

FU-34 **Development of a granular formulation of *Metarhizium brunneum* based on mycelial fragments** Christopher Seib; Johannes Schäfer; Dietrich Stephan, Julius Kühn Institute, Darmstadt Germany

FU-35 **Innovative biological products for soil pest control: Outline of an EU project** Stefan Vidal[1]; Anant Patel[2]; Hermann Strasser[3]; Tariq Butt[4]; Joergen Eilenberg[5]; Juerg Enkerli[6]; Enrique Quesada-Moraga[7]; Justus Wesseler[8]; Francesca Tencalla[9]; Arne Peters[10]; Miloslav Nesrsta[11]; Andrew Shearer[12]; Hermann Limbers[13]; Erik Hansen[14]; Athanasios Koukoutsakis[15]; [1]Georg-August-Universität Göttingen, Germany; [2]University of Applied Sciences, Germany; [3]University of Innsbruck, Austria; [4]Swansea University, United Kingdom; [5]University of Copenhagen, Denmark; [6]Agroscope Reckenholz Tänikon, Switzerland; [7]University of Córdoba, Spain; [8]Technische Universität München, Germany; [9]Toxminds, Belgium; [10]e-nema GmBH, Germany; [11]Fytovita, Czech Republic; [12]Neem Biotech Ltd, United Kingdom; [13]Klasmann-Deilmann GmbH, Germany; [14]EWH BioProduction Aps, Denmark; [15]Torux Software Ltd, UK

FU-36 **Oxidative stress levels in the entomopathogenic fungus *Beauveria bassiana* growing in very long-chain hydrocarbons** Carla Huarte-Bonnet, Nicolás Pedrini, Instituto de Investigaciones Bioquímicas de La Plata (CCT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, Calles 60 y 120, La Plata, Argentina

MICROBIAL CONTROL

MC-1-STU **Fungal strain selection and screenhouse evaluation of the virulent isolate against aphids on crucifer and okra vegetables** Wakuma Bayissa[1,2]; Sunday Ekesi[1]; Godwin P. Kaaya[2]; Samira Mohamed[1]; John M. Wagacha[2]; and Nguya K. Maniania[1], [1]International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya, [2]School of Biological Sciences, University of Nairobi, Nairobi, Kenya

MC-2 **Virulence of fungal spores produced in liquid and solid state media on nymphs of *Trialeurodes vaporariorum*** Eduardo Abreo & Nora Altier; Bio-production Lab, INIA Las Brujas, Canelones, Uruguay

MC-3-STU **Development of entomopathogenic fungi in mosquito control: which kind of production for which efficiency?** Thomas Bawin[1], Frank Delvigne[2], Frédéric Francis[1], [1]Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Belgium, [2]Bio-industries, Gembloux Agro-Bio Tech, University of Liege, Belgium

MC-4 **The basis for rootstock resilient to *Capnodis* species: screening for genes encoding delta-endotoxins from *Bacillus thuringiensis*** Eitan Ben-Dov[1]; Galina Gindin[2]; Zvi Mendel[2]; Arieh Zaritsky[3]; Ariel Kushmaro[4], [1]Department of Life Sciences, Achva Academic College, Israel; [2]Department of Entomology, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel; [3]Faculty of Natural Sciences, Ben-Gurion University of the Negev, Be'er-Sheva, Israel; [4]Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Be'er-Sheva, Israel

MC-5 **Selection of entomopathogenic fungi for the control of *Aegorhynus nodipennis* (Coleoptera: Curculionidae) under laboratory conditions** Ernesto Cisternas[1], Andrés France[2] and Irina Urtubia[2], [1]Instituto de Investigaciones Agropecuarias (INIA), La Cruz, Chile. [2]INIA Quilamapu, Chillán, Chile

MC-6 **Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* strain HD1** Caroline Placidi De Bortoli[1], Ricardo Antonio Polanczyk[1], Neil Crickmore[2], Rafael Ferreira dos Santos[1], Alessandra Marieli Vacari[1] and Sergio Antonio De Bortoli[1]; [1]Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil, [2]Department of Biochemistry, University of Sussex, Brighton, UK

MC-7 **Sublethal effects of the Cry1Ac toxin of *Bacillus thuringiensis* Berliner in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations** Sergio Antonio De Bortoli[1], Caroline Placidi De Bortoli[1], Ricardo Antonio Polanczyk[1], Neil Crickmore[2], Rafael Ferreira dos Santos[1] and Alessandra Marieli Vacari[1], [1]Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil, [2]Department of Biochemistry, University of Sussex, Brighton, UK

MC-8 Effect of *Bacillus thuringiensis* Berliner on biological characteristics of *Orius insidiosus* Say (Hemiptera: Anthocoridae) fed with eggs of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) Sergio Antonio De Bortoli, Ricardo Antonio Polanczyk, Alessandra Marieli Vacari, Roberto Marchi Goulart and Caroline Placidi De Bortoli, Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil

MC-9-STU Evaluating microbial biocontrol agents: effects of *Metarhizium brunneum* on a non-target arthropod Martina Falagiarda, Chad Alton Keyser, Bernhardt M. Steinwender, Lene Sigsgaard, Jørgen Eilenberg, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark

MC-10-STU An experimental autoinoculation device to control an invasive Asiatic pest, *Drosophila suzukii* María Fernández-Bravo, Enrique Quesada-Moraga, University of Córdoba, Department of Agricultural and Forestry Sciences, ETSIAM, Córdoba, Spain

MC-11 Use of a commercial *Metarhizium anisopliae* s.l. formulation to control *Rhipicephalus microplus* ticks in pen study Mariana G. Camargo[1]; Allan F. Marciano[1]; Fillipe A. Sá[1]; Wendell M. S. Perinotto[1]; Simone Quinelato[1]; Patrícia S. Golo[1]; Isabele C. Angelo[1]; Márcia C. A. Prata[2]; Vânia R. E. P. Bittencourt[1], [1]Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil; [2]Embrapa Gado de Leite, Juiz de Fora, MG, Brazil

MC-12 Two Colombian entomopathogenic fungi are highly efficient on *Cerotoma tingomariana* Erika Grijalba; Adriana Santos; Carlos Espinel, Center of Biotechnology and Bioindustry CBB; Colombian Corporation for Agriculture Research, CORPOICA. Mosquera, Colombia

MC-13-STU Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana* Deborah Kaiser[1], Sven Bacher[2] and Giselher Grabenweger[1], [1]Agroscope, Institute for Sustainability Sciences, Zurich, Switzerland, [2]University of Fribourg, Department of Biology, Unit of Ecology and Evolution, Fribourg, Switzerland

MC-14 Pathogenicity and virulence of *Beauveria* spp. against mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytidae) George Kyei-Poku[1], Shajahan Johny[1], William Fick[1], and Katherine Bleiker[2], [1]Great Lakes Forestry Centre, Canadian Forestry Service, Natural Resources Canada, Sault Ste. Marie, Ontario, Canada, [2]Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, Victoria, British Columbia, Canada

MC-15 The Use of Microbial Plant Protection Agents for Insect Control in Germany Johannes A. Jehle, Annette Herz, Brigitte Keller, Regina G. Kleespies, Eckhard Koch, Andreas Larem, Annegret Schmitt, Dietrich Stephan, Julius Kühn Institute, Darmstadt, Germany

MC-16-STU Synthesis and secretion of volatile organic compounds by *Triatoma infestans* infected with *Beauveria bassiana* Luciana S. Lobo[1,2], Sergio J. Mijailosky[1], M. Patricia Juárez[1], Christian Luz[2], Éverton K. K. Fernandes[2] and Nicolás Pedrini[1], [1]Instituto de Investigaciones Bioquímicas de La Plata (CCT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, La Plata, Argentina; [2]Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Brasil

MC-17 Preliminary studies of entomopathogenic microorganisms present in Latvian population of horse-chestnut leaf miner *Cameraria ohridella* Zane Metla[1,2], Rita Seskena[1], Santa Voitkane[1], Monika Maurhofer Bringolf[2], Liga Jankevica[1], [1]Laboratory of Experimental Entomology, Institute of Biology, University of Latvia, Latvia, [2]Plant Pathology, Institute of Integrative Biology (IBZ). Swiss Federal Institute of Technology, Switzerland

MC-18 Toxicity of *Bacillus thuringiensis* BERLINER Cry toxins in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations Ricardo Antonio Polanczyk[1], Caroline Placidi De Bortoli[1], Neil Crickmore[2], Rafael Ferreira dos Santos[1], Alessandra Marieli Vacari[1] and Sergio Antonio De Bortoli[1], [1]Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil, [2]Department of Biochemistry, University of Sussex, Brighton, UK

MC-19 *Bacillus thuringiensis* isolation from Brazilian soil samples: molecular characterization and biological activity against *Plutella xylostella* (Lepidoptera: Plutellidae) Ricardo Antonio Polanczyk[1]; Thiago Trevisoli Agostini[1]; Lais Fernanda Moreira[1], Rogério Teixeira Duarte[1]; Fernando Hercos Valicente[2], [1]Microbial Control of Pests Lab, Plant Protection Department, Universidade Estadual Paulista, Jaboticabal, Brazil, [2]EMBRAPA Milho e Sorgo, Sete Lagoas, Brazil

MC-20 STU Effect of endophytic *Beauveria bassiana* on herbivore defence in *Arabidopsis thaliana* Maya Raad, Travis Glare, Michael Rostás, Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand

MC-21-STU Pathogenicity of *Beauveria* and *Metarhizium* to the two stink bug species *Nezara viridula* and *Piezodorus guildinii* (Hemiptera: Pentatomidae) in laboratory and semi-field Yordanys Ramos González[1], Ingeborg Klingen[2], Jorge R. Gómez Sousa[3], [1]Universidad Central "Marta Abreu de Las Villas (UCLV), Faculty of Agricultural and Animal Science, Villa Clara, Cuba; [2]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Aas, Norway

MC-22 STU Evidence for synergies between *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae) and *Metarhizium brunneum* (Hypocreales: Clavicipitaceae) in western corn rootworm control Hannes Rauch[1,2], Hermann Strasser[1], Roland Zelger[2], [1]Institute of Microbiology, Leopold-Franzens University Innsbruck, Innsbruck, Austria; [2]Research Centre for Agriculture and Forestry Laimburg, Laimburg Auer/Ora, Italy

MC-23 Evaluation of the effectiveness of the entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat Gadi V.P. Reddy[1], Khanobporn Tangtrakulwanich[1], Shaohui Wu[1], John H. Miller[1], Victoria L. Ophus[1], Stefan T. Jaronski[2], [1]Western Triangle Agricultural Research Center, Montana State University, Conrad, USA; [2]United States Department of Agriculture, Agricultural Research Service, Northern Plains Agricultural Research Laboratory, Sidney, USA

MC-24 STU Using the combination of entomopathogenic

fungi and extracts improves control of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) Gloria Resquín-Romero, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga; University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain

MC-25 STU **Wireworm control with fungus colonized barley kernels in cover-crops** Sina Rogge; Giselher Grabenweger, Agroscope, Institute for Sustainability Sciences, Zurich, Switzerland

MC-26 **A resource efficient method to test non target effects of new biocontrol agents in vitro** Bernhardt M. Steinwender, Jørgen Eilenberg, Elina Panahi, Kiri M. Fløistrup, Marta M. Cáceres, Gabriela M. Vergara, Lene Sigsgaard; Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark

MC-27 **Ultrastructure of midgut of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) after consumption of prey with the *Bacillus thuringiensis* strain HD1** Alessandra Marieli Vacari, Vanessa Fabíola Pereira de Carvalho, Caroline Placidi De Bortoli, Ricardo Antonio Polanczyk and Sergio Antonio De Bortoli, Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil

MC-28 **Control of sugarcane borer, *Diatraea saccharalis*, with formulations of *Beauveria bassiana* and *Metarhizium anisopliae*** Inajá M. Wenzel[1,2]; Antonio Batista Filho[2]; Moacir R. Forim[1]; Isabella B. Giordano[1]; Bárbara E. Denadae[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-29-STU **Identification and functional analysis of two ABCC family genes in *Helicoverpa armigera*** Yutao Xiao, Kongming Wu, The State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

MICROSPORIDIA

MI-1 **Decline of native bumblebees (*Bombus*) and *Nosema* (Microspordia: Nosematidae) infections associated with introduction of the European bumblebee in Northern Japan** Maki N. Inoue, Takahiro Yanagisawa, Madoka Nakai, Yasuhisa Kunimi, Institute of Agriculture, Tokyo University of Agriculture and Technology, Japan

MI-2 **Development and application of a loop-mediated isothermal amplification method for rapid detection of *Nosema ceranae*** George Kyei-Poku, Debbie Gauthier, Shajahan Johny, Great Lakes Forestry Centre, Canadian Forestry Service, Natural Resources Canada, Sault Ste. Marie, Ontario, Canada

MI-3 **Permanent level of pathogens within ten bark beetles generations** Karolina Lukášová; Jaroslav Holuša; Jiří Trombik, Department of Forest Protection and Entomology, Faculty of Forestry and Wood Science, Czech University of Life Sciences, Prague, Czech Republic

MI-4 **Microsporidia in beet webworm *Loxostege sticticalis* (Pyraloidea: Crambidae): a survey of 2013** Julia Malysh, Yuri Tokarev, Andrei Frolov, Anastasia Ignatieva, Irma Issi, All-Russian Institute of Plant Protection, St. Petersburg, Russia

MI-5 **Microsporidia from larvae of different lepidopteran species in Bulgaria** Daniela Pilarska[1], Danail Takov[1], Miroslav Hylis[2], Renate Radek[3], Leellen Solter[4], Andreas Linde[5], [1]Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria; [2]Faculty of Science, Charles University, Prague, Czech Republic, [3]Free University of Berlin, Berlin, Germany, [4]Illinois Natural History Survey, University of Illinois, USA; [5]University of Applied Sciences, Eberswalde, Germany

MI-6 **Ultrastructural characterization of a new microsporidium (Opisthokonta: Chytridiopsida) from the pigeon feather mite *Falculifer rostratus* (Astigmata: Pterolichoidea)** Renate Radek[1], Madlen Kariton[1], Jacek Dabert[2], Gerd Alberti[3], [1]Free University of Berlin, Berlin, Germany; [2]Adam Mickiewicz University, Poznan, Poland; [3]Ernst-Moritz-Arndt-Universität Greifswald, Greifswald, Germany

MI-7 **Infectivity of a *Thelohania* like microsporidian isolated from *Phthonandria atrilineata* to the silkworm, *Bombyx mori*** Liangen Shi, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang Province, China

NEMATODES

NE-1 **First release of the mermithid *Strelkovimermis spiculatus* in *Culex pipiens* mosquito populations in Argentina** Evangelina Muttis[1]; María F. Achinelly[2]; María V. Micieli[3]; [1]Fellowship CONICET Centro de Estudios Parasitológicos y de Vectores, CEPAVE, La Plata, Argentina; [2,3]Researcher CONICET, Centro de Estudios Parasitológicos y de Vectores, CEPAVE, La Plata, Argentina

NE-2 **Increased infectivity in Steinernema websteri IJ after development in desiccation-stressed hosts** Andrea Binnebose and Susan M. Bornstein-Forst; Marian University, Fond du Lac, WI 54935 USA

NE-4-STU **Characterization of symbiotic bacteria *Photorhabdus luminescens* subsp. *laumondii* associated with *Heterorhabditis bacteriophora* isolated from Turkey** Harun Çimen; Selçuk Hazır, Adnan Menderes University Faculty of Arts and Science Department of Biology, Turkey

NE-5 **Pathogenicity of nematobacterial complexes and its development** Pavel Dobes; Jakub Berka; Jana Hurychova; Libor Vojtek; Pavel Hyrsl, Department of Animal Physiology and Immunology, Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

NE-6 **Use of entomopathogenic nematodes to control vine weevils on Chilean berry orchards** Andrés France[1], Ernesto Cisternas[2], Irina Urtubia[1], [1]Instituto de Investigaciones Agropecuarias (INIA), Quilamapu, Chillán, Chile, [2]INIA La Cruz, La Cruz, Chile

NE-7 **Nematodes of large larch bark beetle *Ips cembrae***

(Coleoptera: Scolytinae) Sarka Grucmanová[1]; Václav Čermák[2], Jaroslav Holuša[1], [1]Czech University of Life Sciences Prague; Czech Republic, [2]Central Institute for Supervising and Testing in Agriculture, Olomouc, Czech Republic

NE-8 Natural Occurence of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) in the Aydin district of Turkey Baris Gulcu[1], Canan Hazir[2], Mehmet Karagoz[3], M. Alper Kesici[3], [1]Düzce University, Faculty of Arts and Science, Department of Biology, Düzce, Turkey; [2]Aydin Vocational School of Health Services, Adnan Menderes University, Aydin, Turkey; [3]Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, Aydin, Turkey

NE-9 Detection of dsRNA virus-like molecules in entomopathogenic nematodes Noemi Herrero; Jiří Nermuť; Vladimír Půža; Zdeněk Mráček, Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i. Institute of Entomology, České Budějovice, Czech Republic

NE-10 Celullar and humoral interactions between the white grub, *Polyphylla adspersa* Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes Jamileh Avandi[1], Javad Karimi[1], Mohammad Ghadamyari[2] & Ahmad Asoode[3], [1]Biocontrol and Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran, [2]Department of Plant Protection, College of Agriculture Science, University of Guilan, Rasht, Iran, [3]Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Iran

NE-11 *Oscheius rugaolensis*, new genus and species of insect parasitic nematodes from Iran Reyhaneh Darsouei & Javad Karimi, Biocontrol and Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

NE-12 Reproduction status of *Tribolium castaneum* affects its response to infection by *Steinernema feltiae* Dariusz Małek[1], Joanna Homa[2], Maria Gaweł[1], Paulina Kramarz[1], [1]Institute of Environmental Sciences, Jagiellonian University, 30-387 Krakow, Poland, [2]Institute of Zoology, Jagiellonian University, 30-387 Krakow, Poland

NE-13 Effect of culture type, container type, and temperature on a Korean strain of the entomopathogenic nematode, *Steinernema carpocapsae* DongWoon Lee[1]; Ho Yul Choo[2], [1]Major of Applied Biology, School of Ecological Environment and Tourism, Kyungpook National University, Sangju, Republick of Korea; [2]Department of Applied Biology, College & Institute of Agriculture & Life Sciences, Gyeongsang national University, Jinju, Republick of Korea

NE-14 *Steinernema feltiae* (Nematoda: Steinernematidae) to control fungus gnat, *Bradysia mabiusi* (Diptera: Sciaridae): effect of dosage and application time* Patricia Ballone[1]; Luis G. Leite[1]; Fabio S. Schmidt[2]; Victória R. Campos[1]; Roselaine N. S. Bueno[1]; [1]Instituto Biológico, CEIB, CP70, Campinas, Brazil, [2]Bio Controle, Indaiatuba, SP 13347-630, Brazil

NE-15 The non-sterilizing strain of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) and its development on different strains of *Amylostereum* (Basidiomycota: Russulales) Isis A. L. Caetano, Ann E. Hajek, Department of Entomology, Cornell University, Ithaca, New York, USA

NE-16 Use of entomopathogenic nematodes in the biological control of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) Manana Lortkipanidze, Oleg Gorgadze, Madona Kuchava, Nana Gratiashvili, Mzia Kokhia, Nino Gabroshvili, Institute of Zoology, Ilia State University, Tbilisi, Georgi

NE-17 The susceptibility of Colorado potato beetle *Leptinotarsa decemlineata,* and mulberry moth *Glyphodes pyloalis* to entomopathogenic nematodes, *Steinernema carpocapsae* and *Steinernema feltiae* in Georgia Nona Mikaia, Sokhumi State University, Tbilisi, Georgia

NE-18 Co-infection interactions between entomopathogenic fungi and *Steinernema feltiae* using*Tenebrio molitor* as a model system E. Erin Morris, Annette B. Jensen, Anja A. Wynns, Jørgen Eilenberg, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark

NE-19 Some observation on morphology and ecology of mollusc-parasitic nematode *Alloionema appendiculatum* Jiří Nermuť, Vladimír Půža, Zdeněk Mráček; Biology Centre ASCR v.v.i., Institute of Entomology, Branišovská 1160/31, 370 05 České Budějovice, Czech Republic

NE-20 Osmotic stress tolerance and infective juvenile production of entomopathogenic nematodes subject to fast host-desiccation treatments Jaime Ruiz-Vega[1], Teodulfo Aquino-Bolaños[1], Juan R. Delgado-Gamboa[2] and Carlos I. Cortés-Martínez[2], Becarios [1] COFAA-IPN y [2]PIFI-IPN, Laboratory of Biological Control, CIIDIR U. OAXACA, IPN, Santa Cruz Xoxocotlan, Oax., México

NE-21 Assessing entomopathogenic nematode population genetics: a research and teaching approach Abigail Lewis, Logan Jefferson, Glen Stevens, Michaela Gazdik, School of Natural Sciences and Mathematics, Ferrum College, Ferrum, VA, USA

VIRUSES

VI-1 High-level Expression of Foreign Protein Using the Partial Polyhedrin-fused Baculovirus Expression System Sung Min Bae[1]; Tae Young Shin[1]; Jae Bang Choi[1]; Yeon Ho Je[2]; Byung Rae Jin[3]; Soo Dong Woo[1], [1]Department of Agricultural Biology, Chungbuk National University, Chungju, Korea; [2]Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Korea; [3]College of Natural Resources and Life Science, Dong-A University, Busan, Korea

VI-2 A natural recombinant between *S. frugiperda* MNPV and *S. litura* NPV Gloria Barrera[1], Laura Villamizar[1]; Manuel Alfonso Patarroyo[2], Oihane Simón[3], Primitivo Caballero[3], Mariano Belaich[4], Daniel Ghiringhelli[4]; [1]Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia, [2]Fundación Instituto de Inmunología de Colombia (FIDIC), Bogotá, Colombia, [3]Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Navarra, España, [4]Laboratorio de Ingeniería Genética y Biología Celular y Molecular – Area Virosis de Insectos, Universidad Nacional de Quilmes, Argentina

VI-3 Host specificity and PIFs based phylogeny of Betabaculovirus isolates from Gelechiidae family Juliana Gómez[1], Laura Villamizar[1]; Gloria Barrera[1]; Cecilia Turco[2], Mariano Belaich[2], Daniel Ghiringhelli[2], [1]Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia [2]Laboratorio de Ingeniería Genética y Biología Celular y Molecular – Area Virosis de Insectos, Universidad Nacional de Quilmes, Argentina

VI-4 Diagnosing the unknown – advancing the taxonomy of aquatic invertebrate viruses Kelly S. Bateman[1], Grant D. Stentiford[1] and Monique M. van Oers[2], [1]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Dorset, UK, [2]Laboratory of Virology, Wageningen UR, Wageningen, Netherlands

VI-5 Proteomic analysis of the occluded Tipula oleracea nudivirus (ToNV) Annie Bézier[1], Grégoire Harichaux[2], Julien Gaillard[3], Karine Musset[1], Valérie Labas[2], Elisabeth A. Herniou[1], [1]Institut de Recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François Rabelais, France; [2]Laboratoire de Spectométrie de masse, Plateforme d'Analyse Intégrative des Biomolécules et des Phénomique des Animaux d'Intérêt Bio-agronomique. UMR INRA 0085-CNRS 7247-UFR-IFCE, Nouzilly, France; [3]Laboratoire de Biologie Cellulaire, Microscopie Electronique, Faculté de Médecine, Université François Rabelais, Tours, France

VI-6 Nucleopolyhedrovirus and Microsporidia in Winter Moth (*Operophtera brumata*, L.) and Bruce Spanworm (*O. bruceata,* Hurst) populations in the Northeast US Hannah J. Broadley[1,2]; Joseph S. Elkinton[1,2]; John P. Burand[3]; Lina Tian[3]; Leellen F. Solter[4]; [1]Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, USA; [2]Department of Environmental Conservation, University of Massachusetts Amherst, USA; [3]Department of Microbiology, University of Massachusetts Amherst, USA; [4] Department of Entomology, University of Illinois, USA

VI-7 Regulation and activation of two effector caspases that affect Sindbis virus replication in *Aedes aegypti* mosquitoes Ning Huang, A. Lorena Passarelli, and Rollie J. Clem, Division of Biology, Kansas State University, Manhattan, KS

VI-8 Proteomic analysis and *in vivo* diferential gene expression of *Trichoplusia ni* granulovirus (TnGV) Angeles Bivián Hernández; Ingrid Zanella-Sainz; Paloma Dávila-Alvarez, J. Eleazar Barboza-Corona; Fabiola León-Galván; M. Cristina Del Rincón-Castro, Food Department, Division of Life Sciences, University of Guanajuato, Irapuato, Gto. México

VI-9 Recombinant Iridovirus IIV-6 expresing the Cn-10 neurotoxin from *Centruroides noxius* scorpion Flor C. Arellano-Villagómez[1]; Jorge E. Ibarra[2]; M. Cristina Del Rincón-Castro[1], [1]Food Department, Division of Life Sciences, University of Guanajuato, Irapuato, Gto. México, [2]CINVESTAV-IPN Unidad Irapuato, Irapuato, Gto. México

VI-10 Genomic sequencing and analysis of Sucra jujuba nucleopolyhedrovirus Xiaoping Liu, Feifei Yin, Zheng Zhu, Dianhai Hou, Jun Wang, Lei Zhang, Hualin Wang, Zhihong Hu, Fei Deng, State Key Laboratory of Virology, Virus Resource and Bioinformatics Center, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China

VI-11 Functional analysis of exonuclease gene (012L) of *Chilo* iridescent virus Yeşim Aktürk Dizman[1,2], Cemal Sandallı[2], Zihni Demirbağ[1] and Remziye Nalçacıoğlu[1], [1]Karadeniz Technical University, Faculty of Sciences, Department of Biology, Trabzon, Turkey, [2]Recep Tayyip Erdoğan University, Faculty of Arts and Sciences, Department of Biology, Rize, Turkey

VI-12 Identification of a new multiple nucleopoly-hedrovirus isolated from the Jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Egypt Regina G. Kleespies[1], Yongjie Wang[2], Said El Salamouny[3], Mona Awad[3], Essam Agamy[3], Ramadan Salama[3] and Johannes A. Jehle[1,2]; [1]Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; [2]Agricultural Service Station Palatinate, Neustadt/Weinstr., Germany; [3]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.

VI-13 A single baculovirus for the production of recombinant Adeno-Associated Virus 8 vectors Lionel Galibert; Aurélien Jacob; Bérangère Bertin; Marjorie Boutin Fontaine; Delphine Bonnin; Christophe Lecomte; Christel Rivière; Otto-Wilhelm Merten Genethon, 1bis, rue de l'Internationale, Evry, France

VI-14 Determining the role of P10 during baculovirus infection through the development of novel mutants in *Autographa californica* multicapsid Nucleopoly-hedrovirus Leo Graves[1], Farheen Raza[1], Sarah L. Irons[1]; Robert D Possee[1,2] & Linda A King[1], [1]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford UK,[2]Oxford Expression Technologies Ltd, Oxford, UK

VI-15 Evaluation of the transcriptional transactivation of betabaculovirus regulatory elements in transformed cell lines by alphabaculovirus transcription factors Santiago Haase[1], M. Leticia Ferrelli[1]; Matías L. Pidre[1]; Alicia Sciocco-Cap[2], Víctor Romanowski[1], [1]IBBM-UNLP-CONICET, La Plata, AR; [2]IMyZA-INTA, Castelar, AR

VI-16 Enhancin Genes of *Lymantria dispar* NPV Do Not Increase Potency Via Metalloprotease Activity Kelli Hoover[1], James Slavicek[2], Algimantas P. Valaitis[2,3], Nancy Hayes-Plazolles[2], and Elizabeth McCarthy[1], [1]Department of Entomology, Penn State University, University Park, PA USA, [2]USDA Forest Service, Delaware, OH USA, [3] Retired

VI-17 A Cypovirus VP5 Displays the RNA Chaperone-like Activity that Destabilizes RNA Helices and Accelerates Strand Annealing Jie Yang, Jiamin Zhang, Yuehua Kuang and Yuanyang Hu, State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China

VI-18 A recombinant *Autographa californica* nucleopolyhedrosis virus expressing a Cyt1A/GFP chimera in *Trichoplusia ni* larvae Miguel A. Salas-Marina[1], Cristina Del Rincón-Castro[2] and Jorge E. Ibarra[1]; [1]CINVESTAV-Irapuato, Irapuato, GTO, Mexico; [2]División de Ciencias de la Vida, Universidad de Guanajuato, Irapuato, GTO., Mexico

VI-19 iLOV baculovirus: Using a novel small fluorescent protein for imaging virus proteins during infection Farheen Raza[1], Sarah Irons[1], Leo Graves[1], Stan Botchway[2], Robert Possee[1,3], Linda King[1]; [1]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; [2]Central Laser Facility, STFC, Harwell, UK; [3]Oxford Expression Technologies, Oxford, UK

VI-20 Expression analysis of the *nsd-2* gene encoding the putative densovirus receptor in the midgut Katsuhiko Ito[1], Hiroko Tabunoki[1], Takeshi Yokoyama[1], Keiko Kadono-Okuda[2], [1]Tokyo University of Agriculture and Technology, Tokyo, Japan; [2]National Institute of Agrobiological Sciences, Ibaraki, Japan

VI-21 Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua* Agata K. Jakubowska[1]; Melania D´Angiolo[1]; Rosa M. González Martínez[1]; Anabel Millán Leiva[1]; Arkaitz Carballo[2;] Rosa Murillo[2]; Primitivo Caballero[2]; Salvador Herrero[1]; [1]Department of Genetics, Universitat de València, Dr Moliner 50, 46100 Burjassot, Spain; [2]Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, 31192 Mutilva Baja, Navarra, Spain

VI-22-STU A novel baculovirus-derived promoter with high activity in the Baculovirus Expression System Maria Martinez-Solis[1]; Silvia Gomez-Sebastian[2]; Jose M Escribano[3]; Agata K. Jakubowska[1]; Salvador Herrero[1]; [1]Department of Genetics, Universitat de Valencia, Burjassot, Spain; [2]Alternative Gene Expression S.L. (ALGENEX), Centro Empresarial, Parque Científico y Tecnológico de la Universidad Politécnica de Madrid, Campus de Montegancedo, Madrid, Spain; [3]Departamento de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain

VI-23 Construction and Characterization of a Recombinant Invertebrate Iridovirus Arzu Ozgen[1], Hacer Muratoglu[2], Zihni Demirbag[1], Just M. Vlak[3], Monique M. van Oers[3], Remziye Nalcacioglu[1], [1]Karadeniz Technical University; Faculty of Science, Department of Biology, Trabzon, Turkey; [2]Karadeniz Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Trabzon, Turkey; [3]Laboratory of Virology, Wageningen University, Wageningen, The Netherlands

VI-24 RNA interference and insect-virus interactions David Neunemann, David G. Heckel, Heiko Vogel; Max Planck Institute for chemical ecology, Jena ,Germany

VI-25 Studies on existing and new isolates of *Cryptophlebia leucotreta* granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa John K. Opoku-Debrah[1,4]; Martin Hill[1]; Sean Moore[1,2];Caroline Knox[3], [1]Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa; [2]Citrus Research International, Humewood, Port Elizabeth, South Africa.; [3]Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, South Africa; [4]River Bioscience (Pty) Ltd, Humewood, Port Elizabeth, South Africa

VI-26 Effects of the baculovirus fibroblast growth factor on Sindbis virus replication Wenbi Wu, Rollie J. Clem, and A. Lorena Passarelli, Division of Biology, Kansas State University, Manhattan, USA

VI-27 Sensitivity and vertical transmission of nuclearpolyhedrovirus in various populations of gypsy moth Lymantria dispar Olga Polenogova[1], Alexandr Ilyinykh[1], Dmitriy Kurenschikov[2], Philipp Ilyinykh[3], Elena Imranova[2], Alexandr Baburin[2]; [1]Institute of Systematics and Ecology of Animals Siberian Branch of Russian Academy of Sciences, Novosibirsk, RUSSIA; [2]Institute of Water and Ecological Problems Far Eastern Branch of Russian Academy of Sciences Kim-Yu-Chena, Khabarovsk, RUSSIA; [3]State Research Center of Virology and Biotechnology "Vector", Novosibirsk, RUSSIA

VI-28 Establishment of SeMNPV Persistent Infection and Screening of Persistent Infection Associated Genes in Baculovirus Weng Qingbei[1], Li Min[1], Yang Kai[2], Pang Yi[2], [1]School of Life Sciences, Guizhou Normal University, Guiyang, China; [2]State Key Laboratory of Biocontrol and Institute of Entomology, Sun Yat Sen University, Guangzhou, China

VI-29-STU Larvicidal activity of an ascovirus from *Spodoptera litura* against parasitoid wasps Shiori Sagawa, Eiko Arai, Maki Inoue, Yasuhisa Kunimi, Madoka Nakai; Graduate School of Agriculture, Tokyo University of Agriculture and Technology

VI-30 "11K" genes family *sf68*, *sf95* and *sf138* modulate transmissibility and insecticidal properties of *Spodoptera frugiperda* multiple nucleopolyhedrovirus Inés Beperet[1]; Oihane Simón[1]; Trevor Williams[2]; Miguel López-Ferber[3];Primitivo Caballero[1,4]; [1]Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain; [2]Instituto de Ecología AC, Xalapa, Mexico; [3]LGEI, Ecole de Mines d' Alès, Alès, France; [4]Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain

VI-31 Characterization of two ORFs undergoing positive selection in a genotype of *Chrysodeixis chalcites* single nucleopolyhedrovirus from the Canary Islands Oihane Simón[1]; Leopoldo Palma[1]; Alexandra Bernal[1]; Delia Muñoz[2]; Trevor Williams[3]; Primitivo Caballero[1,2]; [1]Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain; [2]Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain; [3]Instituto de Ecología AC, Xalapa, Mexico

VI-32 Genome sequence and organization of a *Betabaculovirus* pathogenicto cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae)
Daniel M. P. Ardisson-Araújo[1]; Fernando Lucas Melo[1]; Miguel S. Andrade[1]; William Sihler[2]; Sonia N. Báo[1]; Bergmann M. Ribeiro[1]; Marlinda L.Souza[2]; [1]Laboratory of Baculovirus, Cell Biology Department, University of Brasília, 70910-900, Brasília, DF;Brazil. [2]Embrapa Genetic Resources and Biotechnology, Biological Station Park, 70770-917, Brasília, DF, Brazil.

VI-33-STU Analysis of genetic interactions among four non-essential genes of BmNPV Hitomi Taka[1], Chikako Ono[2], Masanao Sato[3], Shin-ichiro Asano[1], Hisanori Bando[1]; [1]Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; [2]Research Institute for Microbial Diseases, Osaka University, Suita, Japan; [3]Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, Okazaki, Japan

VI-34-STU Comparative fitness of a granulovirus mutant possessing larger occlusion bodies than wild type *Adoxophyes orana* granulovirus Haruaki Uchida, Yasuhisa Kunimi, Maki Inoue, Madoka Nakai; Graduate School of Agriculture, Tokyo University of Agriculture and Technology

VI-35 Granulovirus detection in larvae of sugarcane borers *Diatraea* spp. (Lepidoptera: Pyralidae) in Colombia Cristian Guzmán, Diana Pinzón, Carolina Ruiz, Juliana Gómez, Carlos Espinel, Gloria Barrera, Laura Villamizar; Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia

VI-36 Earthworm-mediated dispersal of baculovirus occlusion bodies in soil: a laboratory study
Dennis A. Infante-Rodríguez[1]; Delia Muñoz[2]; Jorge Valenzuela[1]; Trevor Williams[1]; [1]Instituto de Ecología AC, Xalapa, Mexico; [2]Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain

VI-37-STU Effects of rearing temperature on the susceptibility of larvae of the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae) to *A. honmai* nucleopolyhedrovirus Takeshi Yamaga, Madoka Nakai, Maki Inoue, Yasuhisa Kunimi, Laboratory of biological control, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu city, Tokyo, Japan

VI-38 Characterization of Nodaviral Protein A Revealed RNA Synthesis and Terminal Nucleotidyl Transferase Activity Zhaowei Wang, Xi Zhou, Dong Li and Congyi Zheng; State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China

SIP Division Business Meeting: Wednesday evening
BACTERIA + Workshop 20:00-21:30. **P5**
Non-Target Effects on Biological Pesitcides Transgenic Crops
Moderator: Ken Narva

199 The impact of herbicide tolerant crops on non-target organisms Ramon Albajes; Marina S. Lee; and Agnès Ardanuy, Universitat de Lleida, Agrotecnio Center, Lleida, Catalonia, Spain

200 Your Right to Know What You Eat: On the Occurrence of Viable *Bacillus thuringiensis* in Commercial Food Products Brian Federici, Department of Entomology and Interdepartmental Graduate Programs in Microbiology & Cell, Molecular and Developmental Biology, University of California, Riverside, Riverside, California USA

201 Environmental risk assessment of genetically engineered crops for spiders Michael Meissle, Jörg Romeis, Agroscope, Institute for Sustainability Sciences, Zürich, Switzerland

202 Conclusions from 10 years of accumulated evidence from publicly funded field trials research with Bt-maize in Germany Stefan Rauschen, Forschungszentrum Jülich GmbH, Projektträger Jülich, Jülich, Germany

SIP Division Business Meeting: Wednesday evening
MICROSPORIDIA + Workshop 20:00-21:30. **P4**

SIP Division Business Meeting: Wednesday evening
FUNGI 20:00-21:30. **P2**

SIP Division Business Meeting: Wednesday evening
VIRUSES 20:00-21:30. **P3**

THURSDAY - 7 August

7:30-16:30 **REGISTRATION** **P1**

Symposium 7 (Dis. of Ben. Invertebr.) Thursday, 8:00 -10:00. **P2**

Emerging Tools for Aquatic Pathogen Discovery and Description

Organizers/Moderators: Spencer Greenwood and Grant Stentiford

8:00 **203 Early mortality syndrome is an infectious disease with a bacterial etiology** Loc Tran[1,2,3], Kevin Fitzsimmons[2] and Donald V. Lightner[1], [1]Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ 85721, USA, [2]Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ 85721, USA, [3]Department of Aquaculture Pathology, Nong Lam University at Ho Chi Minh, Vietnam

8:30 **204 Policy, phylogeny, and the parasite** Grant D. Stentiford[1,2,] Stephen W. Feist[2], David M. Stone[2], Edmund J. Peeler[2] and David Bass[3]; [1]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, UK, [2]Aquatic Pests and Pathogens Group, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset, UK, [3]Division of Genomics and Microbial Diversity, Department of Life Sciences, Natural History Museum, Cromwell Road, London, UK

9:00 **205 The Next Generation of Crustacean Health: Disease Diagnostics Using Modern Transcriptomics** K. Fraser Clark[1,2,3]; Spencer J. Greenwood[1,2;]; [1]Atlantic Veterinary College Lobster Science Centre; [2]Department of Biomedical Sciences, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada; [3] Department of Plant and Animal Sciences, Dalhousie University, Truro, Nova Scotia, Canada

9:30 **206 Environmental DNA as a tool for detection and identification of aquatic parasites: known unknowns and just plain unknowns** Hanna Hartikainen[1,5]; Grant D. Stentiford[2,3]; Kelly Bateman[2,3]; Stephen W. Feist[3]; David M. Stone[3]; Matt Longshaw[3,4]; Georgia Ward[1]; Charlotte Wood[1]; Beth Okamura[1] and David Bass[1]; [1]Department of Life Sciences, The Natural History Museum, London, UK; [2]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset, UK; [3]Aquatic Pests and Pathogens Group, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset, UK; [4]Fish Vet Group, Inverness, [5]ETH Zürich and Eawag, Duebendorf, Switzerland

Contributed Papers Thursday, 8:00-10:00. **P4**

Nematodes 3

Organizer/Moderator: Luis Leite and Glen Stevens

8:00 **207 The Role of biocontrol agents within IPM of *Tuta absoluta* on tomato in Egypt** Mahfouz Abd-Elgawad, Phytopathology Department, National Research Center, Giza, Egypt.

8:15 **208 Insecticidal activity of *Heterorhabditis bacteriophora* Shandong toward *Brontispa longissima* and *Cryptothelea variegate*** Cheng Bai*, Liping Liu, Haibo Long, Qian Jin and Zhengqiang Peng; Key Laboratory of Pests Comprehensive Governance for Tropical crops, Ministry of Agriculture, Hainan Key Laboratory for Monitoring and Control of Tropical Agricultural Pests, Hainan Engineering Research Center for Biological Control of Tropical Crops Diseases and Insect Pests, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan China.

8:30 **209 Prospects for using Entomopathogenic Nematodes to Control the Vine Mealybug, *Planococcus ficus*, in South African Vineyards** Patrique D. Le Vieux, Antoinette P. Malan; Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, Matieland, South Africa.

8:45 **210 New data on *Steinernema ichnusae* distribution in the Mediterranean Area** E.Tarasco[1], M. Clausi[2], G. Rappazzo[2], M. Oreste[1], L. Rubino[2], D. Leone[2], M. T.Vinciguerra[2], [1]Departement of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari "Aldo Moro", Bari (Italy), [2]Department of Biological, Geological and Environmental Sciences, Section of Animal Biology "M. La Greca", University of Catania, Italy

9:00 **211-STU Evaluation of entomopathogenic nematodes for control of the diapausing overwintering codling moth population** Odendaal Deidré; Addison F. Matthew; Malan P. Antoinette; Department of Conservation Ecology and Entomology, Faculty of AgriSciences, University of Stellenbosch, South Africa

9:15 **212-STU A new entomopathogenic *Oscheius* (Nematoda: Rhabditidae) from Italian cave** Giulia Torrini[1], Beatrice Carletti[1], Giuseppe Mazza[1], Pio Federico Roversi [1] , Elena Fanelli[2], Francesca De Luca[2], Alberto Troccoli[2], Eustachio Tarasco[3], [1]Agricultural Research Council - Agrobiology and Pedology Research Centre (CRA-ABP), Firenze (Italy); [2]Istitute of Plant Protection (IPP)-CNR, Bari (Italy); [3]Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari "A.Moro", Bari, Italy

9:30 **213 Genetic improvement of the entomopathogenic nematode *Heterorhabditis bacteriophora*** Ralf-Udo Ehlers, e~nema, GmbH, Schwentinental, Germany

9:45 **214-STU Perspectives of new nematode formulation technology for biological control to pest insects in Georgia** Mariam Chubinishvili, Tsisia Chkhubianishvili, Manana Kakhadze, Iatamze Malania, Kanchaveli Institute of Plant Protection, Agricultural University of Georgia, Tbilisi, Georgia

Contributed Papers Thursday, 8:00-10:00. **P1**

Viruses 6

Moderator: Adly Abd-Alla and Madoka Nakai

8:00 **215 Interactions between salivary gland hypertrophy virus and tsetse microbiota** Güler Demirbas Uzel[1], Vangelis Doudoumis[2], Antonios Augustinos[1], Gisele Ouedroogo[1], Andrew Parker[1], Drion Boucias[3], Kostas Bourtzis[1], Adly Abd-Alla[1], [1]Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria; [2]Department of Environmental and Natural Resources Management, University of Patras, Agrinio, Greece;[3]Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

8:15 **216 STU Mechanisms of tree-top disease induced by the specialist baculovirus SeMNPV** Yue Han, Stineke van Houte, Vera I.D. Ros, Just M. Vlak and Monique M. van Oers, [1] Laboratory of Virology, Wageningen University, Netherlands

8:30 **217 Temporal proteomics to study virus infection and function in the host cell** İkbal Agah İnce[1]; Sjef Boeren[2], Just Vlak[3], Monique van Oers[3]; [1]Department of Medical Microbiology, Acıbadem University, School of Medicine, Istanbul, Turkey; [2]Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands; [3]Laboratory of Virology, Wageningen University, Wageningen, The Netherlands

8:45 **218 Characterization of an atypical fast-killing asco-virus: *Spodoptera frigiperda* ascovirus 1d (SfAV-1d)** Eiko Arai[1]; Shiori Sagawa[1]; Yasumasa Saito[1]; Xiao-Wen Cheng[2]; Dennis Bideshi[34]; Maki Inoue[1]; Yasuhisa Kunimi[1]; Brian Federici[3]; Madoka Nakai[1], [1]Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; [2]Department of Microbiology, Miami University, Oxford, Ohio, USA; [3]Department of Entomology, University of California, Riverside, USA; [4]California Baptist University, Riverside California, USA

9:00 **219-STU Two nucleopolyhedroviruses isolated from the genus *Adoxophyes* inhibit juvenile hormone (JH) esterase activity but not JH epoxide hydrolase activity** Yasumasa Saito[1,2]; Shizuo G. Kamita [2]; Bruce D. Hammock [2]; Yasuhisa Kunimi [1]; Maki N. Inoue [1]; Madoka Nakai [1], [1] Laboratory of Biological Control, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; [2]Laboratory of Pesticide Biotechnology, Department of Entomology and Nematology, University of California, Davis, USA

9:15 **220 Mechanism underlying virus-induced hyperactive behavior: Substrate identification of the baculovirus protein tyrosine phosphatase** Stineke van Houte, Carmen Embregts, Esther van Andel, Vera I.D. Ros, Just M. Vlak and Monique M. van Oers. Laboratory of Virology, Wageningen University, Wageningen, Netherlands

9:30 **221-STU The genome of a baculovirus isolated from *Lonomia obliqua* (Lepidoptera: Saturniidae) reveals a new transcription terminator factor possible acquired from the host** Clara Wandenkolck Silva Aragão[1]; Bergmann Morais Ribeiro[1]; Fernando Lucas Melo[1]; [1]University of Brasília- UnB- Brazil

9:45 **222 The essential baculovirus protein VP1054 is a hijacked cellular PURα, a nucleic-acid-binding protein specific for GGN repeats** Martin Marek[1], Christophe Romier[1], Lionel Galibert[2], Otto-Wilhelm Merten[2] and Monique M. van Oers[3], [1,2,3]Biologie Structurale Intégrative, Institut de Génétique et Biologie Moléculaire et Cellulaire (IGBMC), UDS, CNRS, INSERM, Illkirch, France ; [2]Laboratory of Applied Vectorology, Généthon, Évry, France; [3]Laboratory of Virology, Wageningen University, Netherlands

Symposium (Special) Thursday, 8:00-10:00. **P5**

DFG Priority Program Host Parasite Coevolution

Organizer/Moderator: Joachim Kurtz

8:00 **223 Escaping parasite manipulation: Apoptosis and host-parasite co-evolution in *Apis mellifera*** Christoph Kurze[1], Oleg Lewkowski[1], Yves Le Conte [2], Claudia Dussaubat [2], Thomas Müller[3], Silvio Erler[1], Per Kryger[4], and Robin F.A. Moritz[1]; [1]Institute of Biology, MLU Halle-Wittenberg, Germany; [2]Abeilles et Environnement, INRA Avignon, France; [3]Department of Internal Medicine IV, MLU Halle-Wittenberg, Germany; [4]Depart-ment of Agroecology, Aarhus University, Denmark.

8:15 **224 Overcoming external immunity: An increase in virulence as a result of host-parasite coevolution in *Beauveria bassiana*** Charlotte Rafaluk[1], Wentao Yang[1], Philip Rosenstiel[2], Hinrich Schulenburg[1] and Gerrit Joop[1,3]; [1]Evolutionary Ecology Genetics, Zoological Institute, Christian-Albrechts-Universitaet zu Kiel, Germany, [2]Institut für Klinische Molekularbiologie, Christian-Albrechts-Universität zu Kiel, Universitäts-klinikum Schleswig-Holstein, Campus Kiel, Germany, [3]Institute for Phytopathology and Applied Zoology, University of Giessen, Gießen, Germany

8:30 **225 Rapid adaptation of *Bacillus thuringiensis* to its nematode host *Caneorhabditis elegans*** Leila Masri[1,2]; Antoine Branca[3], Anna Sheppard[1,4]; Hinrich Schulenburg[1], [1]Dept. Evolutionary Ecology and Genetics, University of Kiel, Germany; [2]Present address: IST Austria, Austria; [3]CNRS-Université Paris-Sud, Orsay, France; [4]Present address: Nuffield Department of Medicine, University of Oxford, Oxford, UK

8:45 **226 Intra-host parasite interactions between co-infecting *Bacillus thuringiensis* strains** Michaela H. Klösener, Joy Bose, Rebecca D. Schulte, Department of Behavioural Biology, University of Osnabrueck, Germany

9:00 **227 Experimental evolution *in silico*: host-parasite coevolution versus parasite adaptation** Jakob Strauß[1], Philip Crain[2], Sultan Beshir[1], Joachim Kurtz[1], Hinrich Schulenburg[3], Arndt Telschow[1]; [1]Westfälische Wilhelms Universität, Institute of Evolution and Biodiversity, Münster Germany; [2]DuPont Pioneer, Delaware USA; [3]Christian-Albrechts-Universität zu Kiel, Department of Evolutionary Ecology and Genetics, Kiel Germany

9:15 **228 Immune priming with *Bacillus thuringiensis* in *Tribolium castaneum*** Joachim Kurtz, Barbara Milutinovic, Robert Peuss, Kevin Knoblich, Hendrik Eggert, Sarah Behrens, Jenny Greenwood, Westfälische Wilhelms Universität, Institute of Evolution and Biodiversity, Münster, Germany

9:30 **229 Rapid reciprocal adaptation between the red flour beetle and *Bacillus thuringiensis* bacteria during experimental coevolution** Barbara Milutinovic & Joachim Kurtz, Institute for Evolution and Biodiversity, Münster, Germany

9:45 **230 Means of fast virulence adaption: the plasmid and prophage equipment of selected *Bacillus thuringiensis* strains** Jacqueline Hollensteiner[1;] Joachim Kurtz[2], Hinrich Schulenburg[3], Heiko Liesegang[1], [1]Georg-August University Göttingen, Institute für Mikrobiologie und Genetik, Germany; [2]Westfälische Wilhelms-Universität Münster, Germany; [3]Christian-Albrechts-Universität Kiel, Zoological Institute, Germany

10:00–10:30 **BREAK**

Thursday, 10:30-12:30. **P1**

SOCIETY FOR INVERTEBRATE PATHOLOGY

Annual Business Meeting

Presiding: Jørgen Eilenberg

12:30–14:00 **LUNCH** Mensa

Symposium 8 (Cross-Divisional) Thursday, 14:00-16:00. **P2**

Host – Pathogen Ecology at the Molecular Level: Gene Regulation and Environment Sensing

Organizers/Moderators:
Christina Nielsen-LeRoux and Elke Genersch

14:00 **231 The *Bacillus thuringiensis* way of life: communicate to kill and survive in the insect host** Didier Lereclus, INRA, UMR1319 - Micalis, La Minière, 78280 Guyancourt, France.

14:30 **232 The interplay of Paenibacillus larvae with honey larvae during infection** Elke Genersch; Anne Fünfhaus; Eva Garcia-Gonzalez; Gillian Hertlein; Lena Poppinga, Institute for Bee Research, Hohen Neuendorf, Germany

15:00 **233 Antimicrobial defense and persistent infection in insects revisited** Jens Rolff, Evolutionary Biology, Fachbereich Biologie, Chemie, Pharmazie , Freie Universität Berlin, Berlin, Germany

15:30 **234 *Vibrio* and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes** Audrey Vanhove[1], Annick Jacq[2], Frédérique Le Roux[3], Tristan Rubio[1], Alexandra Calteau[4], Evelyne Bachère[1], Julie Nicod[1], Agnès Vergnes[1], Astrid Lemire[3], Guillaume Charrière[1] and Delphine Destoumieux-Garzón[1]; [1]Ecology of coastal marine systems, University of Montpellier, France; [2]Institut de Génétique et Microbiologie, Université de Paris Sud, France; [3]Integrative Biology of Marine Models, Ifremer, Université Pierre et Marie Curie. Station Biologique de Roscoff, France; [4]Laboratory of Bioinformatics Analyses for Genomics and Metabolism, Genoscope, Evry, France

Contributed Papers Thursday, 14:00-15:45. **P3**

MICROBIAL CONTROL 4

Moderator: Trevor Jackson

14:00 **235 Establishing the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cucurbits for managing Zucchini Yellow Mosaic Virus (ZYMV)** Lara R. Jaber & Nida' Salem, Department of Plant Protection, Faculty of Agricultural Sciences, The Univ. of Jordan, Amman, Jordan

14:15 **236 Bean plant *Phaseolus vulgaris* endophytically colonized by *Beauveria bassiana* and *Hypocrea lixii* acquires protection against *Liriomyza huidobrensis* (Diptera: Agromyzidae) in the field** Jane W. Gathage, Komivi S. Akutse, Komi K.M. Fiaboe, Sunday Ekesi and Nguya K. Maniania, International Centre of Insect Physiology and Ecology, Nairobi, Kenya

14:30 **237 Colonized plants with entomopathogenic fungi produce mortality in *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae** Gloria Resquín-Romero, Cristina Delso, Carlos Campos, Lola Ortega, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga, University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain

14:45 **238 *Beauveria bassiana* and California strawberries: endophytic, mycorrhizal, and entmopathogenic interactions,** Surendra K. Dara, Division of Agriculture and Natural Resources, University of California, USA

15:00 **239 Perceptions, trust, terminology and influence: What do consumers think about biological control?** Michael Brownbridge and Alexandra Grygorczyk, Vineland Research and Innovation Centre, Vineland Station, Ontario, Canada

15:15 **240 A phylogenetic survey of protistan parasites** David Bass[1], Hanna Hartikainen[2], Cedric Berney[1], Sigrid Neuhauser[1], Georgia Ward[1], Grant Stentiford[3]; [1]Division of Genomics and Microbial Diversity, Department of Life Sciences, Natural History Museum, UK ; [2]ETH Zürich and Eawag, Duebendorf, Switzerland; [3]European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, UK

15:30 **241 *Bacillus thuringiensis* toxins vs baculovirus: differential induction of immune system related genes in *Spodoptera exigua*** Cristina M. Crava, Agata Jakubowska, Salvador Herrero, Baltasar Escriche, Yolanda Bel, Department of Genetics, ERI BIOTECMED, Universitat de Valencia, Burjassot, Spain

Contributed Papers Thursday, 14:00-16:00. **P1**

VIRUSES 7

Moderator: Zihni Demirbag and Mehin Yuan

14:00 **242 Lysine Residues in N-terminal Tail of a Viral Histone H4 are Crucial in Controlling Host Gene Expression** Rahul Hepat, Yonggyun Kim, Department of Bioresource Sciences, Andong National University, Andong, Korea

14:15 **243 Heat-shock protein 90 is a broadly active regulator for baculovirus infection** Shufen Li; Dianhai Hou; Fei Deng; Hualin Wang; Manli Wang; Zhihong Hu, Wuhan Institute of Virology, Chinese Academy of Sciences, P. R. China

14:30 **244 Development and immunity-related microRNAs of the lepidopteran model host *Galleria mellonella*** Krishnendu Mukherjee and Andreas Vilcinskas, Fraunhofer Institute of Molecular Biology and Applied Ecology, Department of Bioresources, Giessen, Germany

14:45 **245 The *sf122* gene of *Spodoptera frugiperda* nucleopolyhedrovirus modulates key aspects of insect-to-insect transmission and post mortem host liquefaction** Inés Beperet[1]; Oihane Simón[1]; Trevor Williams[2]; Sarah L. Irons[3]; Leopoldo Palma[1]; Miguel López-Ferber[4]; Linda A. King[3]; Primitivo Caballero[1,5], [1]Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain; [2]Instituto de Ecología AC, Xalapa, Mexico; [3]Department of Biological and Medical Sciences, University of Oxford, United Kingdom; [4]LGEI, Ecole de Mines d' Alès, Alès, France; [5]Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain

15:00 **246 Effect of a Viral Encoded Protein Kinase on Gene Expression in Amsacta moorei Entomopoxvirus Infected Cells** Hacer Muratoglu[1], Remziye Nalcacioglu[2], Basil Arif[3], Zihni Demirbag[2], [1]Karadeniz Technical University, Faculty of Sciences, Department of Molecular Biology and Genetic, Trabzon, Turkey;[2]Karadeniz Technical University, Faculty of Sciences, Department of Biology, Trabzon, Turkey; [3]Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

15:15 **247 FP25K acts as a negative regulator in the infectivity improvement of AcMNPV Budded viruses** Shufen Li, Manli Wang, Zhihong Hu, Fei Deng, Hualin Wang, State Key Laboratory of Virology, Virus Resource and Bioinformatics Center, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, P.R. China.

15:30 **248 The leucines in the transmembrane domain of *Autographa californica nucleopolyhedrovirus* Ac76 are important for intranuclear microvesicle formation** Denghui Wei, Yan Wang, Xiaomei Zhang, Meijin Yuan, Kai Yang, State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, China

15:45 **249 High-throughput purification of dsRNA against sacbrood virus disease in honey bees *Apis cerana* (Hymenoptera: Apidae)** Jianqing Zhang, Yi Zhang and Richou Han*, Guangdong Entomological Institute, Guangzhou China

16:00–16:30	**Student Business Meeting**	**P4**

18:30	**Bus transfer to SIP Banquet**	Alte Lokhalle

IMPORTANT NOTE: Remove all posters before 18:00

19:00-1:00 RECEPTION

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ABSTRACTS 2014

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B-11 indicates abstract number for POSTER presentation

MONDAY - 4 August

PLENARY SYMPOSIUM Monday, 10:30–12:30

Microbial Control - from Bench to Business

PLENARY SESSION. Monday, 10:30. **1**

Potentials for utilizing and controlling insect pathogens

Richou Han, Xuehong Qiu and Xun Yan
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Insects are attacked by different invertebrate pathogens. Diverse strategies are used to deal with these pathogens. In this presentation, three examples are presented to show the utilization and control of insect pathogens in Guangdong Entomological Institute, China: (1) ***Ophiocordyceps sinensis* fungus as health food.** *O. sinensis* (Clavicipitaceae) (best known as *Cordyceps sinensis*) is one of the entomopathogenic fungi endemic on above 3000 m Tibetan Plateau. The fungus parasitizes larvae of moths (Lepidoptera) and fruiting bodies grow from the infected larvae. Regarded as "Himalayan Viagra", the fungus-insect complex is used to treat a variety of ailments including fatigue, impotence and cancer, and costs $60000–$75000 per kilogram. The growing worldwide demand and resource limitation drive the research to artificial cultivation of this fungus for commercial trade. (2) ***Photorhabdus* bacteria for insect control.** *Photorhabdus* bacteria associated with entomopathogenic *Heterorhabditis* nematodes produce oral protein toxins for killing insects. For sustainable termite control, the toxic genes are transformed into *Enterobacter cloacae*, one of the indigenous gut bacteria of the Formosan subterranean termite (*Coptotermes formosanus*), and the termites are fed with these genetically modified bacteria. (3) **Control of Chinese sacbrood virus (CSBV) by RNAi-mediated technology.** CSBV is the most serious virus of oriental honey bees *Apis cerana*. To protect the honey bees, RNAi technology is successfully used to control this harmful virus, by feeding second instar larvae of *A. cerana* with specific sequences of CSBV double-stranded RNA (dsRNA). The results from these examples show the research strategies in invertebrate pathology and potentials for implementing the research results in commercial purpose.

Key words: Invertebrate pathogens, *Ophiocordyceps sinensis*, *Photorhabdus* bacteria, CSBV
Funding: This work was supported by National Natural Science Foundation of China (No. 31010103912 and 31000879), Guangdong Provincial Science & Technology Project (No. 2012B050700008 and 2010A040301012) and Funding of Guangdong Academy of Sciences for Young Scientist (No. qnjj201301).

PLENARY SESSION. Monday, 11:00. **2**

Story of an African firm: 10 years in the biopesticide business – lessons learned along the way

Sean Moore
Citrus Research International, Port Elizabeth, South Africa; Rhodes University, Grahamstown, South Africa
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In 2003, River Bioscience in South Africa became the first African company to successfully produce and commercialise an insect virus as a biopesticde. This was the *Cryptophlebia leucotreta granulovirus* (CrleGV). The outcome was instant success, mainly due to the good fortune of perfect timing. The target pest, the false codling moth (*Thaumatotibia leucotreta*), was a very serious one and there was a dearth of alternative products. River Bioscience originated as a spin-off company from grower-funded citrus research and for the first few years of existence, served that single agricultural sector as a one-product company: a high risk situation. Subsequently, the company expanded its product range into other viruses, including the *Helicoverpa armigera* nucleopolydrovirus (HearNPV) and the *Cydia pomonella* granulovirus (CpGV), entomopathogenic nematodes and a range of biorational products, such as Attract and Kill products for a range of fruit fly species. The success of the commercial venture can be attributed to a number of factors, including product quality and competitiveness, being market driven rather than product driven, starting small (hence not over capitalizing) and growing organically, a close association with research organisations and being owned by its major market – the citrus growers. However, all has not been moonlight and roses: many hard lessons have been learned. For example, simply having a good product is not sufficient – it is the way in which the product is marketed that determines how it sells relative to the competition, which has increased dramatically since the emergence of the company.

PLENARY SESSION. Monday, 11:30. **3**

A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods

Willem J. Ravensberg
Koppert Biological Systems, Veilingweg 14, 2651 BE Berkel en Rodenrijs, the Netherlands
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Microbial pesticides have been developed for a hundred years, but many of these crop protection products have not been successful in the market. Therefore, there is a need for a model that facilitates the development and commercialization of these products. A model has been developed for a biocontrol product based on entomopathogenic bacteria, fungi, viruses and nematodes. The model aims to develop a rational and structured approach that will increase the chances of achieving success with microbial pest control products. The building blocks of the entire process are identified and essential aspects highlighted. This systematic roadmap with a strong focus on economics and market introduction will assist academic researchers and industrial developers of biopesticides in accomplishing their goal: the development of successful cost-effective biopesticides.

PLENARY SESSION. Monday, 12:00. **4**

BASF Functional Crop Care. Unlocking Agricultural Potential in Soil, Seed and Crop

Sebastian Bachem
BASF – Limburgerhof, Germany
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For many years BASF has been active in the area of biological pest control with its pheromone based mating disruption solutions and in 2012 it acquired the leading biologicals company Becker Underwood. With its broad range of seed,

soil and foliar products Becker Underwood was an excellent fit and has now been fully integrated into the company.
The presentation will outline the different key segments BASF is focusing on in the areas of soil, seed and foliar treatments. Furthermore it will focus on the main opportunities BASF sees in developing an integrated portfolio of biological and chemical products that are able to reliably cover a broad spectrum of farmer's needs. Beyond this we will look forward and outline how we expect the crop protection market to develop and what motivates BASF to invest into finding best possible solutions to meet these changing market demands.

SYMPOSIUM 1 (Nematodes) Monday, 14:00-16:00

Above and Belowground Interaction, Root-Shoot Interaction, Chemical Signaling

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

Small molecule signals in nematodes - common motifs and species specific modifications

Stephan H. von Reuss
Max Planck Institute for Chemical Ecology, Department of Bioorganic Chemistry, Jena, Germany
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Chemical communication in nematodes via small molecule signals has been known since the 1960s. However, despite considerable efforts chemical structures have remained elusive for several decades. Recent research focusing on the model organism *Caenorhabditis elegans* has revealed a modular library of small molecule signals, the ascarosides, glycolipids of the dideoxysugar ascarylose linked to fatty acid derived side chains, that modulate nematode development and behavior. Furthermore, we have shown that production of ascaroside components is highly conserved among nematodes from different clades, life-styles and ecological niches.
Our ongoing research aims to comprehensively characterize ascaroside signaling in selected nematode species including bacteriovorus and entomopathogenic species. Identification of putative ascaroside signals is accomplished using our recently developed highly sensitive HPLC-MS/MS precursor ion screen that facilitates the detection of known and novel ascaroside components in crude nematode metabolome extracts. Novel ascarosides are subsequently isolated by SPE and HPLC and identified using a combination of HR-MS/MS and NMR techniques. We found that diverse nematode species share a large variety of common ascarosides and in addition also produce several highly species-specific derivatives. Chemical synthesis and subsequent functional characterization of these putative small molecule signals in different nematodes will reveal their importance in intra- and interspecific communication and help to decipher the evolution of ascaroside signaling in nematodes.

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

Olfactory Plasticity in Entomopathogenic Nematodes

Joon Ha Lee and Elissa Hallem
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Many parasites, including entomopathogenic nematodes (EPNs), use host-emitted olfactory cues to locate hosts. However, how parasitic nematodes respond to host-emitted odors remains poorly understood. In particular, little is known about how parasitic nematodes integrate host odor cues with environmental cues such as temperature and intrinsic cues such as age to mediate context-appropriate host-seeking behaviors. To address this question, we are investigating the olfactory behavior of EPNs from the genera *Steinernema* and *Heterorhabditis*. We find that EPNs are attracted to the general host cue carbon dioxide under all conditions tested. However, responses to many odorants exhibit extreme olfactory plasticity as a function of IJ cultivation temperature and/or age. For example, in *Steinernema carpocapsae*, many odorants that are strongly attractive at lower temperatures are strongly repulsive at higher temperatures and vice versa. This temperature-dependent olfactory plasticity occurs in individual IJs and is reversible, since temperature-swapping IJs reverses their olfactory preferences. By contrast, other species appear to show primarily age-dependent changes in olfactory preferences, while still other species show little or no olfactory plasticity. Thus, the type and extent of olfactory plasticity varies among EPNs. In addition, we find that foraging strategy can also vary with temperature. For example, *Steinernema carpocapsae* behaves more like an ambusher at higher temperatures and more like a cruiser at lower temperatures. Some EPNs are found in geographical regions that undergo substantial seasonal temperature variation, and we hypothesize that plasticity of olfactory behavior and foraging strategy may enable EPNs to optimize host seeking under changing environmental conditions.

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

Multiple Consequences of Belowground Herbivore Induced Volatile Signals

Jared G. Ali[1,2], Raquel Campos-Herrera[2,3], Hans T. Alborn[4], Larry W. Duncan[2], Lukasz L. Stelinski[2]
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Plants can influence the behavior of and modify community composition of soil dwelling organisms through the exudation of organic molecules. Given the chemical complexity of the soil matrix, soil-dwelling organisms have evolved the ability to detect and respond to these cues for successful foraging. A key question is how specific these responses are and how they may evolve. Soil nematodes are a group of diverse functional and taxonomic types, which may reveal a variety of responses. Herbivore-induced volatile emissions benefit plant hosts by recruiting natural enemies of herbivorous insects. Such tritrophic interactions have been examined thoroughly in aboveground terrestrial environments. Recently, similar signals have been described in the subterranean environment, which may be of equal importance for indirect plant defense. Our work has shown that plant roots of citrus defend themselves against root herbivores by releasing an herbivore-induced plant volatile (HIPV), pregeijerene (1,5-dimethyl-cyclodeca-1,5,7-triene), that attracts naturally occurring entomopathogenic nematodes (EPNs) to larvae when applied in the field. However, the soil community is complex, containing a diversity of interspecies relationships that modulate food web assemblages. In a series of experiments we examine the specificity of this HIPV in the complex nematode community, including beneficial entomopathogenic nematodes, plant-parasitic nematodes, as well as, hyper-parasitic nematodes and nematophagous fungi. We provide the first evidence showing subterranean HIPVs behave much the

same as those aboveground, attracting not only parasitoids, but also hyperparasites and other food web members.

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

Root Zone Chemical Ecology; New Techniques for Below Ground Sampling and Analyses of Volatile Semiochemicals

Hans T. Alborn [1]; Fatma Kaplan[2]
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The ban of the fumigant methyl bromide has led to a need for new methods to control soil-dwelling plant pests. The use of semiochemicals is one such avenue of research since studies of plants above ground release of volatile organic compounds (VOCs) in response to herbivory have resulted in effective control methods for insect pests and also plant roots might release induced VOCs that attract organisms such as entomopathogenic nematodes. However, studies of such below ground interactions lags because of the complexity of the system. For example, in addition to plants roots, potentially important VOCs can be produced also by microorganisms, insects and nematodes and in soil VOCs are released into a virtually static airspace where they disperse solely by diffusion. To bypass this complexity root-related VOCs have been sampled by transferring roots from a pot to an artificial environment where most of the air surrounding the roots is drawn through an adsorption filter that trap VOCs, or by maceration and solvent extraction. This creates an artificial VOC profile with little relevance to the system intended to be studied. To address the need for more sensitive and less intrusive *in vivo* studies of below-ground VOC governed interactions probes were designed for direct in-soil sampling. In combination with improved thermal desorption GC/MS analyses the probes allowed short sampling times and required removal of minimal air volumes. This technique makes it possible to continuously monitor and follow the dynamics of root zoon VOCs in response to insect or nematode infestations.

CONTRIBUTED PAPERS Monday, 14:00-16:00

BACTERIA 1

Contributed paper. Monday, 14:00. **9**

Discovery of Insecticidal Proteins from Non-Bacillus Bacterial Species

Nasser Yalpani[1]; Dan Altier[1], Jennifer Barry[1], Jarred Oral[2], Ute Schellenberger[2], Adane Negatu[1], Scott Diehn[1], Virginia Crane[1], Gary Sandahl[1], Joe Zhao[1], Dave Cerf[2], Claudia Perez Ortega[3], Mark Nelson[3], Analiza Alves[1], Lu Liu[2], Gusui Wu[1]
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Crops expressing various *Bacillus thuringiensis*-derived insecticidal Cry protein genes have been on the market for over 15 years and have provided significant value to growers. Such products also provide a significant positive impact on the environment due to the reduced need for chemical insecticides. However, there remains the need for the discovery of alternative actives that can complement or substitute for Cry toxins. A screen of bacterial collections led to the discovery of several insecticidal protein genes with great potential for developing insect resistant crops. Two examples representing actives from non-Bacillus sources will be presented: PIP-1A is a 30 kD protein isolated form a *Pseudomonas* strain showing strong activity against hemipteran and certain lepidopteran pests. AfIP-1A and AfIP-1B is a pair of binary proteins isolated from an *Alcaligenes* strain demonstrating potent corn rootworm killing activity. Corn plants expressing this pair of proteins display high resistance to WCRW. Preliminary studies on AfIP-1A and AfIP-1B in terms of protein biochemical characteristics, insecticidal activity spectrum and insect mid-gut binding properties indicate this pair of binary proteins may function in ways similar to some Cry proteins from Bacillus sources. Our work demonstrates that bacteria that are not Bacillus can be valuable sources of insecticidal proteins.

Contributed paper. Monday, 14:15. **10**

Discovery and optimization of hemipteran-active proteins for Lygus control in cotton

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The plant bugs *Lygus hesperus* and *Lygus lineolaris* have emerged as economic pests of cotton in the United States. These hemipteran species are not controlled by the lepidopteran-specific insect control traits (*Bacillus thuringiensis* Cry proteins) found in genetically-modified commercial varieties of cotton. We have identified several novel Bt Cry proteins that are toxic to Lygus nymphs in artificial diet bioassays. Several of these proteins have been further modified to exhibit improved toxicity towards both Lygus species while retaining the insecticidal specificity of the parent protein. Cotton plants expressing modified Cry proteins show enhanced protection from Lygus feeding damage in the field.

Contributed paper. Monday, 14:30. **11**

Isolation and identification of potential biological control agent from *Tortrix viridana* L.(Lepidoptera: Tortricidae) pupae

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Tortrix viridana is one of the most important pest in the oak fields in Turkey. The aim of this study is to find a more effective and safe biological control agent against *Tortrix viridana.* For this purpose, pupae of *T. viridana* were collected from Artvin province, Turkey in 2013. According to the morphological, biochemical tests, API20E and API50CH panel test system and 16S rRNA gene sequence analysis, the bacterial isolates were identified as Serratia *liquefaciens* (Tv1), *Enterococcus* sp. (Tv2), *Rhodococcus erytropolis* (Tv3), *Rahnella aquatilis* (Tv4), *Curtobacterium flaccumfaciens* (Tv5), *Pseudomonas* sp. (Tv6). Future research will be tested insecticidal effects of these bacterial isolates against *T. viridana.*.

Contributed paper. Monday, 14:45. **12 STU**

Evolution of a Sensor Protein Controlling Production of an Insecticidal Toxin in Plant-Beneficial *Pseudomonas protegens*

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Pseudomonas protegens is a plant-associated bacterium that is not only able to promote plant growth by efficiently protecting roots from attack by fungal phytopathogens but also can turn into an insect pathogen. The microorganism is capable of killing certain pest insects upon oral infection. The major goal of our work is to understand the molecular mechanisms that allow *P. protegens* and related bacteria to detect, to survive within and to kill the insect host. The entomopathogenic activity of *P. protegens* relies in part on the production of an insecticidal toxin termed Fit. We found that the pseudomonad produces the Fit toxin in the insect host, but not on plant roots, demonstrating that the bacterium is capable of distinguishing between these two environments. An array of sensor proteins makes bacteria able to sense the environment they live in and to adapt their behavior accordingly. Here we provide evidence that the sensor histidine kinase FitF is a key regulator of insecticidal toxin production. Our experimental data and bioinformatic analyses indicate that FitF shares a sensing domain with DctB, a histidine kinase regulating carbon uptake in Proteobacteria. This suggests that FitF has acquired its specificity through domain shuffling from a common ancestor. This particular event appeared to be crucial for host-dependent activation of toxin production and thus contributed to the evolution of insect pathogenicity in these bacteria. We propose that inhibition of the FitF sensor during root colonization is the underlying mechanism by which *P. protegens* differentiates between the plant and insect host..

Contributed paper. Monday, 15:00. **13 STU**

***Paenibacillus larvae*, the etiological agent of American Foulbrood, produces the catechol type siderophore bacillibactin**

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The Gram positive, spore forming bacteria *Paenibacillus larvae* is the causative agent of American Foulbrood, a fatal disease affecting the brood of honey bees. The whole vegetative life cycle of *P. larvae* takes place inside the larvae and all micronutrition must be obtained from the host- including iron- a scarce atom essential for growth of host and pathogen likewise. Bacteria often answer this iron deficiency with the production of siderophores, small molecules which act as powerful iron chelators. Such siderophores are often synthesized by mulitenzyme complexes through non-ribosomal peptide-synthetases (NRPS). The genes of these multienzyme complexes are arranged in giant gene clusters.
Here we present data on the identification of an NRPS gene cluster in *P. larvae* encoding the biosynthetic machinery for the production of a siderophore, which was identified as bacillibactin by MS/MS. Exposure bioassays with mutant *P. larvae* strains lacking bacillibactin production showed that neither total mortality nor disease progression in infected larvae was significantly changed compared to larvae infected with the corresponding wild-type strain. These results are in line with results published on the role of bacillibactin in other pathogenic bacteria like *Bacillus thuringiensis* and *B. anthracis*.

Contributed paper. Monday, 15:15. **14**

Two new *Bacillus thuringiensis* toxins active against Lepidoptera and Coleoptera.

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The entomopathogenic spore-forming bacteria, *Bacillus thuringiensis* (Bt), is widely distributed around the world and is able to produce toxins with insecticidal activity during the vegetative and sporulation phase. The great genetic variety of *B. thuringiensis* strains represents a huge diversity of potential insecticidal proteins. The host range of these proteins is highly variable, but includes a large number of species of the most damaging lepidopteran insect pests and also, other harmful species of the orders Diptera, Coleoptera and Hymenoptera. In order to extend the number of Bt proteins active against important coleopteran and lepidopteran pests, total DNA of a strain from a Spanish collection was completely sequenced. Two ORFs of ~900 bp were selected due to their low identity with other Bt proteins and were cloned in a Bt expression plasmid. Proteins were produced and their insecticidal activity was determined. Bm_47 protein was toxic against *Leptinotarsa decemlineata*, with an LC_{50} of 54 µg/ml, while Bm_1711 protein was active against the lepidopterans *Helicoverpa armigera* and *Ostrinia nubilalis*, with an LC_{50} of 164 and 34 ng/cm^2, respectively. We discuss the importance of this protein to combat species of coleopteran and lepidopteran pests, including species that have developed resistance to other Bt toxins..

Contributed paper. Monday, 15:30. **15-STU**

Entomopathogenic *Bacillus thuringiensis* as PGPR

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Bacillus thuringiensis has been used as an effective bio-insecticide because it produces the proteins Cry and Cyt, which are highly toxic to insects in certain situations. But, recently *B. thuringiensis* was treated as a biological control agent which could control the plant disease. We already demonstrated that the antagonistic activity of *B. thuringiensis AS17 japonensis, AS18 kurstaki* against *Fusarium oxysporum* f.sp. *lycospersici* race2 (FOL) was examined by dual culture technique(Qi et al. 2013). In this study, *B. thuringiensis* strains could control the development of wilt symptoms caused by FOL in tomato plants was confirmed. Inoculate six

strains of *B. thuringiensis* suspension to the tomato seedlings in pot, and transplanted the treated tomato seedlings to FOL infested soil, after 4 weeks the development of wilt symptoms and wilting score become less than control, especially *B. thuringiensis AS17 japonensis* and *AS20 CR371-H*. Also, this study proved that *B. thuringiensis* strains are PGPR. PGPR (Plant growth promoting rhizobacteria) are beneficial bacteria which have the ability to colonize the plant roots and either promotes plant growth through direct action or via biological control of plant diseases. Six strain of Insect Pathogenic *Bacillus thuringiensis* were tested for PGPR effect. Culture filtrates of six strains had remarkable plant growth promotion activity in tomato and alfalfa plants; in each plant after treatment of culture filtrates, both of seed germination rates and the fresh weight were increased compared with control treatment.

Contributed paper. Monday, 15:45. **16**

Vibrios pathogenic for oysters are found associated to plankton species. What possible consequences on pathogen transmission to oysters?

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Vibrios cause major losses in shellfish farming and are associated to recurrent mortalities of oysters. However, to date, the role of plankton species in the transmission of pathogenic vibrios in oyster *Crassostrea gigas* is largely unknown. The main objective of the present study was to identify *in situ* and *in vitro* the interactions of pathogenic Vibrios with local species of planktons from different sites of Thau lagoon, an important region for shellfish farming in south of France. Quantitative-PCR was used to monitor *Vibrio splendidus* and *Vibrio aestuarianus* over the year 2013 at two sites of the Thau lagoon. Out of the oyster farm area, *V. splendidus* was found from May to July and from June to August associated to 5-180 µm and >180µm plankton fractions, respectively. *V. aestuarianus* was also detected in fraction 5-180 µm in May and >180µm in August, before and after the warmer months of the year. For the farm oysters point, *V. splendidus* was found in January and June associated with the 5-180 µm plankton and with the >180 µm fraction in spring and winter. *V. aestuarianus* was not detected. In laboratory controlled conditions, by using a GFP-expressing *V. splendidus* LGP32 and epifluorescence microscopy, we showed that *V. splendidus* LGP32 exhibits strong interactions with copepods of the *Acartia* and *Paracartia* genius as well as with microalgae of the *Alexandrium* genius. Altogether, our data show that vibrios pathogenic for oysters can establish close associations with plankton species, which may enhance the transmission of pathogenic vibrios to oysters.

VIRUSES 1

Contributed paper. Monday, 14:00. **17**

Investigation of Baculovirus RNA Polymerase Subunit Protein-Protein Interactions with *in vivo* Bimolecular Fluorescence Complementation Assays

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Baculovirus transcription utilizes two different DNA-directed RNA polymerases (RNAPs): the insect host RNAP transcribes early genes while a virus RNAP transcribes late and very late genes. The virus RNAP consists of four proteins: P47, LEF-4, LEF-8 and LEF- 9. Conserved motifs in LEF-8 and LEF-9 suggest that the interface of these subunits forms the catalytic site of the RNAP, while LEF-4 has RNA capping-associated enzymatic activities. No specific function has yet been demonstrated for P47. To investigate the *in vivo* intracellular localization and interactions of these proteins, two individually non-fluorescent fragments (V1 and V2) of the Venus yellow fluorescent protein were fused with the N-termini of each RNAP subunit in plasmid expression vectors. We also constructed similar fusions with two components of the virus replisome complex, LEF-3 and P143, and of the host *Spodoptera frugiperda* TATA binding protein. Bacmids, expressing each of these fusion proteins, were constructed and used to generate recombinant viruses expressing each of the V1- or V2-tagged protein subunits. Protein-protein interactions of these subunits were investigated using bimolecular fluorescence complementation assays. Co-infections were used to investigate the interactions of these subunits in the presence of the full complement of virus proteins. Reciprocal co-transfections of the original plasmid constructs were performed to investigate the potential for these proteins to form homo-oligomers, as well as their ability to interact with heterologous partners in the absence of any other viral proteins. The results of co-transfection and co-infection assays will be presented.

Contributed paper. Monday, 14:15. **18-STU**

Characterization and Quantitative Analysis of *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV) FP25K Localization and Aggregate Formation During Cell Infection

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Localization of AcMNPV FP25K was previously studied by western blot using fractionation. This study, however, was not quantitative. By inactivating the endogenous *fp25k* gene through passage of the AcBacmid in Sf21 cells and subsequent insertion of an *fp25k-egfp* fusion gene at the *polyhedrin* locus, we investigated FP25K localization during infection. Western blot confirmed the 53-kDa FP25K-EGFP fusion protein from infected cells. By using a nuclear stain, we were able to assess and quantify the nuclear to cytoplasmic localization of FP25K-EGFP during Hi5 and Sf9 cell infection through confocal microscopy. During late phase of infection, small aggregates were formed and FP25K-EGFP was found exclusively in the cytoplasm. However, during very late phase of infection, larger aggregates were observed in both the

cytoplasm and nucleus and about 1% of FP25K-EGFP localized to the nucleus. In addition, bioinformatic analysis of FP25K predicts a highly conserved coiled-coil domain at the N-terminus. We hypothesize that this coiled-coil domain may be responsible for the formation of these amorphous aggregates in the cytoplasm and nucleus. Therefore, disruption of the coiled-coil domain will disorder aggregate formation. Quantifying FP25K localization and studying aggregate formation may help to understand the role of FP25K aggregates in infection and polyhedrin promoter activities.

Contributed paper. Monday, 14:30. **19 STU**

Bracovirus-derived genes in the genome of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) and their role in host susceptibility to pathogens

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The association between parasitic wasp, its polydnavirus and the lepidopteran host can represent an interesting model to study the horizontal transfer of genes since three different genomes are simultaneously in contact. In this context, the transcriptome of *Spodoptera exigua* revealed the presence of eight unigenes with high homology to bracovirus genes. All of them encoded for lectin-like proteins except one coding for a protein with homology to proteins of unknown function, which we named *gasmin*. Sequence analysis of the genomic region of *gasmin* and of one of the bracovirus lectin-like proteins (*Se-BLL2*) confirmed their integration into the *S. exigua* genome. Gasmin as well as the lectins were mainly expressed in the hemocytes which indicate their possible role in the interaction with the parasitic wasp and insect's immune response. Functional analysis of gasmin revealed that this protein interacts with the cellular actin inhibiting its polymerization. This inhibition leads to a drastic reduction in the capacity of hemocytes to phagocytise bacteria. Moreover, high expression of gasmin reduces the multiplication and the production of baculovirus particles in cell culture experiments. Analysis of the bracovirus-derived lectins revealed that they respond to gram-positive and gram-negative bacteria in addition to baculovirus infection. Remarkably, *Se-BLL2* responds to all tested pathogens. Further characterization of Se-BLL2 showed that it recognizes and agglutinates gram-negative as well as gram-positive bacteria. The results obtained suggest that the insect has domesticated the viral genes to cope with infections by pathogens.

Contributed paper. Monday, 14:45. **20**

Entry of *Bombyx mori* nucleopolyhedroviruse (BmNPV) into BmN Cells by Macropinocytic Endocytosis

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Abstract: *Bombyx mori* nucleopolyhedroviruse (BmNPV) is a serious virus pathogen of silkworm, *Bombyx mori*, and no drugs or specific protection is available presently, whereas knowledge on BmNPV entry, a remarkable target for the development of protection target, is still limited. Here we used BmNPV virus combined with different drugs and subcellular analysis to investigate BmNPV entry mechanism. Results indicated that BmNPV entry into BmN cells was clathrin- and caveolar/lipid raft- indpedent endocytosis pathway, but actin-, microtube-, PKC-, Rac1- and PI(3)K-depedent, and virus entry mediated by cholesterol in a dose dependent manner, these results suggested that BmNPV entry into BmN cells by macropinocytic endocytosis, which was further confirmed by TEM and live image analysis. Our study suggested that BmNPV take a different mechanism to invade host cell that was different with that of AcMNPV..

Contributed paper. Monday, 15:00. **21**

Nuclear translocation of *Autographa californica* nucleopolyhedrovirus ME53

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The baculovirus early/late *me53* is conserved in all sequenced lepidopteran baculovirus genomes. If AcMNPV *me53* is deleted, DNA replication is normal but virus replication and spread is severely compromised. The 449 amino acid AcMNPV ME53 is a nucleocapsid-associated protein colocalizing with the major envelope glycoprotein GP64 at putative "budding" foci on the cell membrane. However, ME53 also localizes to the nucleus. In the absence of an easily identifiable nuclear localization signal we wished to identify ME53 sequences responsible for its nuclear translocation. To that end we generated a series of HA- or GFP- tagged N and C-terminal and internal deletions of ME53 as well as internal ME53 peptides through a baculovirus bacmid intermediate. Localization of the tagged ME53 variants following bacmid transfection, was monitored by confocal fluorescence microscopy. An HA-tagged ME53 lacking aa83-152 was excluded from the nucleus while an internal HA-tagged aa83-152 peptide showed nuclear localization. Further N-terminal deletions up to aa107 (or carboxy terminal deletions up to aa250) showed nuclear localization of GFP-tagged ME53, while N-terminal deletions up to aa121 did not. Among several internal deletions tested, the aa107-121 deletion lacked nuclear localization. Overlapping that region was an alphahelical domain aa107-133. However alanine mutagenesis of some of the basic residues (E121A, R122A, K126A and even double E121A/R122A) predicted to destroy the alphahelix, failed to prevent nuclear localization. As the aa83 to 152 peptide on its own showed nuclear localization we predict the ME53 nuclear localization domain to begin between aa107 and 121 and end upstream of aa152.

Contributed paper. Monday, 15:15. **22**

Nuclear localization and other domains of *Autographa californica* nucleopolyhedrovirus DNA polymerase

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The baculovirus *dnapol* is a core gene essential for viral DNA replication. The 984 aa AcMNPV DNApol has polymerase and 3'-5' exonuclease domains spanning aa189-750. To determine if these domains were sufficient for viral DNA replication and virus production we generated a series of bacmids with DNApol C-terminal deletions. Virus spread and DNA replication were monitored following transfections using GFP

fluorescence. Deletion of the C-terminal 184 aas was detrimental to virus production, and even deletion of the C-terminal 36 aas severely compromised virus spread. Thus almost the entire C-terminus beyond the polymerase domain was required for normal virus replication. Confocal fluorescence microscopy showed this might be due to failure of DNApol nuclear localization. Of several expression plasmids with C-terminal DNApol truncations fused to EGFP, only pBC949, expressing DNApol aa1-949 translocated to the nucleus; shorter truncations remained cytoplasmic, mimicking the results for the same truncations in bacmid constructs. AA sequences in aa804-827 and aa939-948 were consistent with a bipartite and monopartite NLS, respectively. Peptides with either NLS fused to EGFP, independently allowed for strong nuclear localization. However, deletion of either NLS in DNApol:EGFP fusions resulted in only cytoplasmic DNApol:EGFP. A highly conserved C-terminal sequence at aa972-981 was found in all group I alphabaculoviruses. For bacmid constructs with alanine mutagenesis in this region, there was limited spread of GFP fluorescence but only by 144 hpt. Thus DNApol requires both NLSs and even the C-terminal 10 aas for nuclear translocation, viral DNA replication and virus production.

Contributed paper. Monday, 15:30. **23-STU**

Investigations into the role of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) AC141 (EXON0) and *Trichoplusia ni* kinesin-1 in budded virus nucleocapsid egress

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The nucleocapsids (NC) of alphabaculoviruses budded virus (BV) virions are assembled in the nuclei of infected cells, transported from the nucleus, through the cytoplasm and bud from the plasma membrane enabling systemic spread of infection. The AcMNPV viral protein AC141 (EXON0) is required for efficient BV production and has been shown to associate with β -tubulin and potentially directly interact with a Drosophila kinesin-1 TPR domain. The objective of this study was to determine if AC141 can associate with the host lepidopteran kinesin-1. To enable these studies the sequence of *T. ni* kinesin-1 heavy (KHC) and light (KLC) chains were identified from a transcriptome analysis of *T. ni* Tnms42 cells. *T. ni* KLC and KHC cDNAs were subsequently generated and cloned into plasmid expression vectors, and tagged at the 5' and 3' ends with Myc or HA epitope tags, or EGFP. These constructs were used to generate stably transformed High Five (BTI-Tn5B1-4) cell lines. Initial experiments showed that both N- and C-terminal HA-tagged KLC expressed in stable cell lines co-immunoprecipitates AC141 and β-tubulin. In addition, HA-tagged AC141 co-immunoprecipitates with WT KLC. Sequential confocal laser scanning microscopy shows that Myc-KLC in stable cell lines co-localizes with HA-AC141 in regions adjacent to the plasma membrane at 20, 24 and 48 hpi. This technique was also used to examine co-localization of AC141, microtubules and tagged KLC molecules. These studies provide additional support to a model in which the association of AC141 with microtubules plays an important role in anterograde trafficking of BV NCs
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Contributed paper. Monday, 15:45. **24**

The Twist In Baculoviruses

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It has been know for over forty years that baculovirus genomes are supercoiled ds DNA molecules, yet the implications of this fact has had little affect on current explanations of baculovirus replication and hyperexpression. It is now known that negatively supercoiled ds DNA is spontaneously bound by nucleosomes upon entering the nucleus, which is what happens to baculovirus genomes on nuclear entry. Because both replication and transcription require that nucleosomes be slid or removed for these processes to occur, baculoviruses also must be able to regulate chromosome remodeling. Two of the four major classes of chromasome remodelers, INO80 and SWI/SNF, contain actin as an essential subunit. If either or both were necessary for transitioning from late to very late gene expression, the observed transient dependence on polymerizable actin for the period of transition would be explained. Moreover, it is now known that replication of covalently-closed circular DNA in eukaryotic systems requires topoisomerase II (topo 2). Topo 2 makes double-strand breaks (DSBs) and DSB's are considered to be among the most deleterious of DNA lesions. Their occurrence could explain the induction of the DNA damage response during baculovirus replication. SWI/SNF complexes facilitate topo 2 positioning for dsDNA cleavage, hence polymerizable actin is also required. An SV40-based model of baculovirus replication will be presented.

CONTRIBUTED PAPERS Monday, 14:00-15:30

FUNGI 1

Contributed paper. Monday, 14:00. **25**

A new mycopesticide developed especially for the control of the citrus greening vector *Diaphorina citri* (Hemiptera: Liviidae)

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The citrus greening also known as Huanglongbing or yellow dragon disease is one of the most serious citrus diseases in the world. This disease has devastated millions of hectares of citrus crops throughout Brazil and the United States. Considering that once infected the plant has no cure, the primary control strategies currently employed requires intensive use of chemical insecticides against the vector, *Diaphorina citri*. We have developed a new suspension concentrate formulation based on *Isaria fumosorosea* for controlling this pest. The product is effective against adults and nymphs of *D. citri* but it can also contribute to the management of other citrus pests such as the black citrus aphid, *Toxoptera citricida*, the citrus blackfly, *Aleurocanthus woglumi*, and the snow scale, *Unaspi citri.* The *I. fumosorosea* isolate used presented UV tolerance up to two times higher than other fungal isolates tested, and it is compatible and can be tank mixed with most chemicals sprayed in citrus

(pesticides, foliar fertilizers, adjuvants) except for the fungicides. Field sprays (n= >15) on adults confined in voile bags on commercial citrus groves using 60mL of suspensions (2.5-5.0 x 10^6 conidia/mL) per m^3 of leaf area in the citrus canopy caused total mortality ranging from 60-96%. Transmission of the fungus from *D. citri* and *T. citricida* cadavers to uninfected *D. citri* were effectively demonstrated in laboratory and semi-field conditions. The mycopesticide is currently in preparation for commercial registration.

Contributed paper. Monday, 14:15. **26**

Effectiveness of biorationals and *B. bassiana* against tomato fruitworm in Sinaloa

Cipriano García [1], Adolfo D. Armenta [1] and Luis A. Gaxiola [1]
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During autumn-winter 2012 was conducted a field trial with applications of biorational products *B. thuringiensis* (Versa™), Pyirethrins (Abatec™), *S. carpocapsae* (Capsanem™) and native strains of *B.bassiana* and water as control, against neonate larvae of tomato fruitworm *Heliothis virescens* (Fabricius) in a tomato crop cultivated in Guasave Sinaloa, México. The variables evaluated were larvae mortality (LM), fruit damage and yield. The better treatments were: *B. thuringiensis* 39.6%, Pyirethrins 32.3 % and *S. carpocapsae* 23.33%, while the native *B. bassiana* strains ($2.1x10^6$ spores/ml) had 6.3 to 6.6%, and the control 2.66% of LM after 72h. Not statistical differences were found in fruit damage between Versa and Abatec, but they were found in the control and Bb1 strain; in the yield, neither were founded differences between biorational products, these also showed the highest fruit yields, followed by Bb strains. These results indicated a lower field efficacy of fresh native Bb strains at this spore concentration, respect to the other products against *H. virescens*.

Contributed paper. Monday, 14:30. **27**

Evaluating Metarhizium brunneum F52 Microsclerotia Applied in Hydromulch for Control of Asian Longhorned Beetles

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The entomopathogenic fungus Metarhizium brunneum F52 (Hypocreales: Clavicipitaceae) is able to produce environmentally persistent microsclerotia. Incorporating these desiccation-tolerant microsclerotia (Mb MS) granules into hydromulch [a mixture of water + wheat straw mulch + psyllium tackifier], represents a novel, easy-to-use and environmentally-friendly mycoinsecticide that can be sprayed onto the trunks of forest or orchard trees to control insect pests. Hydromulch holds moisture that allows microsclerotia to germinate, and the production of conidia in turn, causes lethal infections in Asian longhorned beetles, Anoplophora glabripennis Motschulsky (Coleoptera: Cerambycidae). To test how quickly beetles could be killed, moist and dry bark pieces and filter paper were sprayed with a low dose (~9 Mb MS/cm^2) of microsclerotia in hydromulch. Median survival times of beetles exposed to moist bark and filter paper were 17.5 d and 19.5 d, respectively. Beetles exposed to dry bark died significantly slower. In an attempt to kill beetles faster, moist bark pieces were sprayed with three doses of microsclerotia in hydromulch: low (6-9 Mb MS/cm^2); medium (10-19 Mb MS/cm^2) and high (20-30 Mb MS/cm^2). At high doses, 50% of beetles died in 12.5 d but at lower doses it took significantly longer to kill beetles (16.5 d-17.5 d). In a two week oviposition period, total beetle fecundity was highest in high-humidity controls, females produced 18.5 viable offspring compared to high-humidity hydromulch treatments that significantly reduced fecundity to 7.9 viable offspring. This however was not significantly different from the low-humidity hydromulch (8.1 viable off spring) or that associated control (9.1 viable offspring). Outdoor spore production by microsclerotia, using moist bark pieces, sprayed with a high dose of hydromulch (20-30 Mb MS/cm^2) and attached to trees in the woods was quantified. There was a significant increase in spore production over 24 days and in the second replicate (another 24 days) spore production was significantly higher than in the first replicate ($P \leq .0001$). Importantly, rainfall was significantly correlated ($P \leq .0042$) to this increase in spore production. Environmental moisture plays a big role in the spore production by microsclerotia and will subsequently affect the level of insect mortality.

Contributed paper. Monday, 14:45. **28-STU**

Management of entomopathogenic fungal disease in rearing mealworms, *Tenebrio molitor* as animal feed

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Mealworm, Tenebrio molitor L. (Coleoptera: Tenebrionidae) has high and safe protein contents, which enables it to be animal feed. However, occurrence of entomopathogenic fungi in mealworms is one of the limitations for mass production. In this work, we investigated relationships between abiotic conditions and occurrence of fungal pathogens and established an effective control method using fungicides. In virulence assay, third instar mealworm larvae were sprayed by six entomopathogenic Beauveria bassiana isolates and kept under high relative humidity; B. bassiana ERL1575 isolate had highest virulence. Under normal humidity, ERL1575 conidian showed different virulence between spray (~0% virulence) and digestion (~80% virulence) method. Furthermore, mealworms, which digested conidia, were exposed to various temperature (20-35°C) and humidity (1-3 ml distilled water spray/35 mm diam. dish) conditions for 5 days. All the treatments showed ~90% virulence except 35°C incubations (~20% virulence), but irrespective to the humidity conditions. Forty chemical fungicides were assayed against conidial germination and hyphal growth of ERL1575. Fluazinam and mancozeb showed strong inhibition of conidial germination at standard application dose (SD), 1/2 SD and 1/5 SD; besides, fluazinam showed strong inhibition of hyphal growth. When fluazinam and mancozeb were applied to the fungal conidia-inoculated wheat bran, most of mealworms were alive after 3 days post application. However, high mortality rate (~100%) were observed in the conidia-inoculated wheat bran without any fungicides. In conclusion, this work suggests that B. bassiana isolates could be pathogens at <30°C when they were digested by mealworms, and fluazinam and mancozeb would be used as effective control agents against the pathogen.

Contributed paper. Monday, 15:00. **29**

Use of *Beauveria bassiana* (Bals) in the management of larger grain borer, *Prostephanus truncatus* (Horn.) (Coleoptera: Bostrichidae) on stored maize in Tanzania

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Maize (*Zea mays* L.) is important for livelihoods in sub-Saharan Africa as it is the major staple food for the majority of people. In Tanzania 82 % of all farms, 4.5 million farmers in total, produce maize. The greater proportion of the maize (98 %) is produced by resource poor farmers, on an average of 0.8 hectares, in remote villages with poor road networks and post- harvest storage facilities which often make them incur high post-harvest losses. Grain loss in Africa due to insect pests' damage in storage systems is estimated at 20 to 30 %. The larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), a native to meso-America, is known to cause considerable economic losses of up to 48% dry weight. While satisfactory control of LGB has been obtained by use of synthetic pesticides in Tanzania, since its accidental introduction in the late 1970s, their adverse effects on environment, possible development of resistance and residues in food have motivated the search for safer alternative methods. One such strategy is the use of biological control using entomopathogenic fungi such as *Beauveria bassiana* (Bals.-Criv.) Vuill. The current paper presents the findings of an ongoing laboratory study to evaluate the efficacy of a formulation (8.65×10^8 CFU g^{-1} spore powder) of *B. bassiana*, isolate IMI 389521 against unsexed adult LGB and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in Tanzania.

Contributed paper. Monday, 15:15. **30**

Management of *Frankliniella occidentalis* (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi

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Western flower thrips (WFT), *Franklinella occidentalis*, is a major pest of ornamentals. Mycotized millet grains with entomopathogenic fungi applied to soil of potted marigold plants was tested to target pupating thrips. Two experimental fungal isolates, (*Beauveria bassiana* [ARS7060] and *Metarhizium anisopliae* [ERL1171]), were compared with the registered *B. bassiana* strain GHA [commercialized as BotaniGard®] and untreated controls in greenhouse caged trials. Mycotized millet grains were mixed into the upper surface of the potting soil in pots of flowering 'Hero Yellow' marigolds (4 g/pot). One week after application five mated WFT females were released onto each plant (four plants per cage). At 8 wks post-infestation, the mean total number of thrips per plant was 81 and 90% less in the ERL1171 and ARS 7060 treatments, respectively, than in the controls. The mean numbers of thrips per plant for the control and GHA treatments were not significantly different. Plant damage was 60% less on plants treated with the experimental fungi than the control and GHA treatments. At 10 wks post-application, 75-90% of WFT collected from the treatments were infected with the experimental isolates. These results demonstrate that soil applications of entomopathogenic fungi can reduce WFT populations significantly and prevent damage.

SYMPOSIUM 2 (Microsporidia) Monday, 16:30-18:30

Microsporidiology: Advances in Europe

Symposium. Monday, 16:30. **31**

A new intracellular parasite is a missing link between fungi and microsporidia

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Intracellular obligate parasitism results often in extreme adaptations, whose evolutionary history are difficult to understand, because intermediate forms are hardly ever found. Microsporidia belong to an early-diverging clade of fungi, which evolved extreme physiologic and genomic simplification as well as exceptionally high rates of molecular evolution. They possess the smallest eukaryotic genomes with very few introns, short intergenic regions and bacterial-sized ribosomal genes. As observed in other eukaryotic intracellular parasites, mitochondria in microsporidia have degenerated into small double-layered organelles called mitosomes, which have lost the genome and cannot produce ATP anymore. Instead, they steal it from their hosts. We describe the evolutionary history of a gut parasite of the crustacean *Daphnia* with remarkable morphological similarity to the microsporidia, but genomic features of ancient fungi. This parasite, which we formally name *Mitosporidium daphniae* gen. et sp. nov., possesses mitochondria, genes for oxidative phosphorylation and an infection apparatus typical for microsporidia. Phylogenomics places *M. daphniae* together with the microsporidia in a clade that also includes the most ancient fungi, the Cryptomycota. Comparative genomics further supports the missing link status of *M. daphniae* highlighting both its microsporidian and fungal like characteristics, and reveals the intermediate evolutionary steps that led to extreme metabolic simplification. The new species demonstrates that the extreme reduction in energy metabolism genes as well as the loss of introns in microsporidia was preceded by a reduction in the machinery controlling cell cycle, DNA recombination, repair and gene expression that may have contributed to the characteristically accelerated rate of microsporidia evolution..

Symposium. Monday, 17:00. **32**

Parasite takes fly - A Drosophila model of Microsporidia infection

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More than 150 years of Microsporidia research led to a basic understanding of many aspects of microsporidial biology, yet little is known about the genetic basis and molecular mechanisms of the intimate host-parasite relationship that govern Microsporidia infections. Genetic model organisms such as *Drosophila melanogaster* are relevant to the study of human infectious disease as most disease-associated genes have homologues in the fly genome. The knowledge about *D. melanogaster* host defense against obligate intracellular parasites remained, however, particularly patchy for lack of good infection models. A few years ago, a strain of *Tubulinosema ratisbonensis* infested our laboratory fly cultures and led us to model Microsporidia infections in *Drosophila*. Thus, we developed the first infection model of parasitism by a eukaryotic intracellular parasite of *Drosophila*, *T. ratisbonensis*. A unique feature of the *Drosophila* model is that we have developed infection models both in permissive cell lines and in adults. In addition, we have identified several nonpermissive cell lines that will allow us to identify some host defense genes. The ease to move from insights gained at the cellular level from *Drosophila* cell cultures to the whole-organism level using transgenic techniques will allow gaining an in-depth understanding of the biology of Microsporidia in flies, especially when combined with multi-'omics' and functional genomic approaches that we have started to implement. This infection system provides thus novel opportunities to understand the mechanisms underlying microsporidiosis in other invertebrate such as bees and vertebrates hosts and may hopefully lead to novel concepts relevant to parasitology.

Symposium. Monday, 17:30. **33**

White Sea metchnikovellids: morphology, life cycles; potential ancestral features of microsporidia

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Family Metchnikovellidae (Class Rudimicrosporea Sprague 1977) seemingly a basal taxon of Microsporidia, remains understudied. We present data on ultrastructure of two species of metchnikovellids infecting lecudinid gregarines from polychaetes *Pygospio elegans* sampled in the White Sea silt littoral zone. The first species, *Metchnikovella incurvata,* Caullery and Mesnil was described in 1914, the second, *M. spiralis* -- only recently (Sokolova et al., in press). The two species have similar structure of free spores, vary in intracellular development, and produce dissimilar spore sacs (cysts). The cysts of the latter species exhibit unusual morphology: they are limited by a thick electron dense wall, externally ornamented with spirally wound cords of dense material. Basing on comparison of fine morphology and life cycles of metchnikovelllids and other microsporidia, I believe that the following traits could be treated as plesiomorphic among microsporidia: paired nuclei, meiosis, division by internal budding (endoplygeny), short or anisofilar polar filaments, and sequence producing thick-walled environmental cysts. Metchnikovellidean spores possess short polar filaments (manubria) and likely do exploit the mechanism of dispersion via everting the polar tube with the attached sporoplasm, the major synapomorphy of Microsporidia. At the same time metchnikovellidan spores are devoid of most elements of the extrusion apparatus: a polaroplast, posterior vacuole, rigid spore wall, and long polar filament connected with a polar disc. The minimal apparatus of metchnikovellids may allow dissemination only within one cell (autoinvasion), whereas production of thick-walled cysts enables horizontal transmission of spores among hosts. .

Symposium. Monday, 18:00. **34**

Microsporidia: Pathogens of Opportunity

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Kudo published the first comprehensive treatise on the microsporidia "A Biologic and Taxonomic Study of the Microsporidia" in 1924 which was a critical review and systematic treatment of all literature on the microsporidia at the time. It would be more than 50 years before another treatise would be produced, "Biology of Microsporidia" in 1976 and Systematics of the Microsporidia" in 1977 by Vavra and Sprague. This would remain the "go to" authority on the microsporidia until 1999 when "The Microsporidia and Microsporidiosis" was published containing chapters on microsporidia in vertebrate and invertebrate hosts and the first comprehensive review of the growing field of molecular biology and phylogeny of the microsporidia. With the field rapidly advancing in many aspects of basic and molecular biology of the microsporidia it was apparent that a revision and expansion of the previous volume was needed. This effort has resulted in the Microsporidia: Pathogens of Opportunity L.M. Weiss and J., J. Becnel (Eds.) John Wiley & Sons, Oxford, UK with 25 chapters complied by experts including evolutionary and molecular biologists, veterinarians, entomologists, ichthyologists and physicians who study microsporidia. This is intended as a resource for those students and young researchers with an interest in the study of microsporidia as well as expanding the knowledge base of microsporidiologists from different disciplines within the field. An overview of the various chapters will be presented and topics of current relevance highlighted.

NEMATODES 1

Contributed paper. Monday, 16:30. **35**

Measuring entomopathogenic nematode activity, abundance and soil food web assemblage in Swiss wheat and maize cultivation

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Wheat and maize are major crops in Switzerland. As part of a research consortium that explores ways to improve soil health, we study how entomopathogenic nematodes (EPNs) can be better exploited for the biological control of soil-dwelling insect pests. We evaluated the impact of different agricultural management practices on native EPN populations in two 30-years Swiss field trials. One experiment compared tillage versus no-tillage and monoculture (wheat) versus crop rotation (maize), whereas the second studied four levels of tillage in two soil types planted with wheat. Soil samples were taken in April and in October 2013 (n = 88). Total nematode activity, as recorded with the *Galleria*-bait technique was <5%, with no significant effect of the treatments. Real time qPCR revealed that >95% of infected cadaver contained a mix of EPN with the competing *Acrobeloides* -group and/or *Oscheius* sp. The available molecular probes identified and quantified 13 organisms from soil, comprising six nematophagous fungi (NF), ectoparasitic bacterium, two free-living nematodes (FLN), and four EPNs (the evaluation of an additional ten EPN species is ongoing). In general, only trace levels of EPN were detected in all soils. *Heterorhabditis* spp. were the dominant EPN, with *H. bacteriophora* being significantly reduced by tillage (P<0.001). Monoculture favored the competitors of EPN (P<0.01). The abundance of EPN, NF and FLN was positively correlated (P<0.05). Since only low numbers of EPN are naturally present in Swiss agricultural soils, an augmentation strategy may help to improve the control of root pests of wheat and maize.

Contributed paper. Monday, 16:45. **36-STU**

Biocontrol and nutrition: understanding the role of environment in the trait deterioration of an entomopathogenic nematode symbiont

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Entomopathogenic nematodes (EPNs; genera *Heterorhabditis* and *Steinernema*) kill their invertebrate hosts with the aid of a mutualistic bacterium. The bacteria (*Xenorhabdus* spp. for steinernematids and *Photorhabdus* spp. for heterorhabditids) are primarily responsible for killing the host and providing the nematodes with nutrition and defense against secondary invaders. EPNs are amenable to laboratory rearing and mass production for biocontrol applications against insects; however, EPNs and their symbiotic bacteria exhibit trait deterioration or changes due to laboratory rearing. The overall goal of this project is to understand how virulence in the nematode-symbiont *Photorhabdus* has evolved in an *in vitro* environment and the role nutrition plays in this process. Nutritional effects in trait deterioration were determined using monoxenic cultures of a freshly isolated strain of *P. luminescens* subsp. *luminescens* where base populations were compared with bacteria that were sub-cultured repeatedly to determine fitness loss. Trait stability was monitored in three different liquid media that are frequently used in laboratory culture: Liquid Lipid Medium, nutrient broth, and trypic soy both + 0.5% yeast extract. Subpopulations were compared to base populations for inclusion bodies after 5,15, and 20 liquid growth cycles (of 48 hours each). Additionally, growth curves and LT_{50} values were determined for the base and deteriorated populations. Differences were observed among the media types and the base/deteriorated populations. Understanding nutritional effects on important biocontrol traits may aid in more efficient methods of mass production.

Contributed paper. Monday, 17:00. **37**

Insect-killing nematodes also kill competitors: lethal male-male fighting in *Steinernema*

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Steinernema spp are well-known as entomopathogenic nematodes. We have found that males of certain species fight and kill each other, and that killing is influenced by the developmental pathway followed. The transmission stage of *Steinernema* is an infective juvenile (IJ) analogous to the dauer juvenile of *Caenorhabditis elegans*. IJs seek out and enter living insects in soil. Inside the insect they release symbiotic bacteria (*Xenorhabdus* spp.) which proliferate and digest the host tissues. This provides a rich nutritive medium for the developing nematodes, which reproduce in the host cadaver. A large host may support several generations of nematodes, and thus represents a valuable resource, worth competing for. In *Steinernema longicaudum*, males of the founding generation (those developing from IJ) fight by wrapping their tail ends around each others' bodies and squeezing. Victims may appear paralysed within minutes of such an encounter, and frequently die. Worms that develop within the host cadaver in second or later generations develop directly, without passing through the IJ stage. For such worms, the benefits of fighting (the quality of the resource) is diminishing, while the large number of rivals present means that the benefits of killing do not necessarily accrue to males that kill. We have found that males that develop directly, without passage through the IJ stage, are much less likely to fight than those that do, and that this appears to be a developmental effect rather than a response to conditions at the time of fighting. .

Contributed paper. Monday, 17:15. **38-STU**

Comparison of Life History Traits of the Entomopathogenic Nematodes *Steinernema feltiae* and *Steinernema riobrave*

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Life history traits (LHTs) of *Steinernema feltiae* and *S. riobrave* were assessed at 25°C using a hanging drop technique. The LHTs were studied with 5 x, 10x and 20x 10^9 cells ml^{-1} of *Xenorhabdus bovienii* and *X. cabanillasii* for *S. feltiae* and *S. riobrave,* respectively, in semi-fluid nematode growth gelrite. The results indicated that increased food density had a significant positive influence on offspring production and net reproductive rate (R_0) on both, *S. feltiae* and *S. riobrave*. Highest offspring production was recorded at bacterial food densities of 20 x10^9 cells ml^{-1} with 813/female for *S. feltiae* and 1,913 offspring/female for *S. riobrave*. Higher R_0 values of 707 and 1,903 were recorded for *S. feltiae* and *S. riobrave,* respectively. A significant positive correlation between bacterial density and body volume that contributed to an increased offspring production was found in both species. The lowest intrinsic rate of natural increase (r_m) (1.1 days) was recorded for *S. feltiae* and the highest (1.4) for *S. riobrave*. A population doubling time of PDT = 0.6 days was recorded for *S. feltiae* and 0.5 days for *S. riobrave*. The life span of female nematodes was not significantly different among the bacterial food densities tested. Significant differences in offspring production and population growth rate were recorded between the two species. The result can be used to further investigate the optimal bacterial food density for mass production in bioreactors for maximum DJ recovery in liquid bacterial suspension, synchronised population development and DJ yields of *S. feltiae* and *riobrave*.

Contributed paper. Monday, 17:30. **39 STU**

How does plant domestication influence entomopathogenic nematodes as potential biological control agents?

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We are studying the effects of plant domestication on belowground plant induced responses to herbivore feeding and how this affects entomopathogenic nematode (EPN) populations. In the New Jersey Pinelands, natural populations of highbush blueberries (*Vaccinium corymbosum*) are commonly found adjacent to commercial fields of domesticated highbush blueberries. In a 3-year field study, we found that EPN were more prevalent but less diverse in cultivated fields than in natural stands. The dominant species in both habitats was *Steinernema glaseri* (*Sg*): numerous isolates of two distinct *Sg* strains were identified. In laboratory studies with oriental beetle (*Anomala orientalis*) larvae, the dominant root-feeder in cultivated fields, *Sg* blueberry isolates were less virulent than the *Sg* NC strain, and strains from cultivated fields tended to be more virulent than those from natural stands. We are using the same *Sg* strains in laboratory and field studies on EPN attraction to blueberry roots as affected by oriental beetle feeding. Ongoing studies suggest that *Sg* is attracted more strongly by damaged roots. We have yet to identify any herbivore induced plant volatile (HIPV) responsible for enhanced attraction. A comparison of 2 known HIPVs emitted from roots in other systems suggests that (E)-β-caryophyllene is more attractive than pregeijerene.

Contributed paper. Monday, 17:15. **40**

Analysis of intraspecific variability in *Steinernema kraussei* populations using PCA

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Species determination in Entomopathogenic Nematodes of the genera *Steinernema* and *Heterorhabditis* is a very complex task, given the broad variability of both morphological and morphometric traits within a single species. To accomplish that, molecular techniques have been adopted which however require additional knowledge. Particularly relevant would be the possibility of testing in a reliable way the variability between different populations of the same species, which might represent different strains with different biological properties. Aim of our work was to determine if morphometric analysis, performed using the "Principal Component Analysis" approach, was able to get evidences of characters with significant diagnostic value, allowing to make reliable distinctions among strains. Four strains of *Steinernema kraussei* were found in Italy, three from Sicily and one from Alps (Tarasco *et al*., 2014;doi:10.1017/S0022149X14000194). Morphometric analysis of morphological traits commonly used in nematode taxonomy (referred to as *variables*) was done on 20 juveniles, males and females of first and second generations (or *observations*) belonging to three strains: 3D and PL (Sicily) and BT (Alps). Statistics was done by SIMCA package v.13. Up to three components were routinely computed; score plots, loading plots, X/Y overview and contribution plots were obtained. Our results showed that some of the morphometric variables employed could reliably be used to discriminate both juvenile and adult forms of PL strain, whereas an insufficient distinction could be made between BT and 3D.

Contributed paper. Monday, 17:30. **41**

Population genetic structure of entomopathogenic nematode *Steinernema affine* (Steinernematidae: Nematoda) inferred using microsatellite markers

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Population genetic structure of entomopathogenic nematodes is still poorly understood even though such knowledge could help us to assess stability and vulnerability of natural EPN populations. Molecular markers used in EPN taxonomy and phylogeny (ITS and LSU regions of rDNA, NAD4, COII) are too conservative to be used to assess within species variability. So far only few studies attempted to use AFLP method to investigate EPN intra-population variability. In present study, microsatellite markers for *Steinernema affine* were developed. In total 218 bioinformatically validated pairs of primers for various oligonucleotides were obtained. Thirty most promising oligonucleotides were selected and tested for the use in the study of the species' population genetic structure. Markers showing variability were identified and examined in various populations of *S. affine*, collected mainly in the area of South Bohemia..

Contributed paper. Monday, 17:45. **42 STU**

Eat or Be Eaten: Fungus and Nematode Switch off as Predator and Prey

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The parasitic nematode *Deladenus siricidicola* is widely used for the biological control of the invasive pine-killing woodwasp, *Sirex noctilio*. The nematode has a unique life cycle where it lives in pine trees, feeding on the symbiotic fungus of *S. noctilio*, the basidiomycete white rot fungus *Amylostereum areolatum*. In the presence of *S. noctilio* larvae, however, the nematode develops into a parasitic form which invades the woodwasp larvae, ultimately leading to sterilization of the host. The fungal-feeding stage of the nematode is used to commercially mass produce it for biological control programs. Previous studies investigating the effect of *A. areolatum* strain on *D. siricidicola* reproduction suggested the possibility of a role reversal where the fungus could eat the nematode. The present study examined the relationships between three species of *Deladenus* nematodes and their associated *Amylostereum* fungi. For *D. siricidicola* and *A. areolatum*, we hypothesized that significantly fewer nematode eggs placed in petri dishes containing potato dextrose agar medium would hatch in the presence of *A. areolatum* fungus than in control petri dishes with no fungus. Results supported this hypothesis. Additionally, light microscopy, fluorescence microscopy, and cryogenic scanning electron microscopy were used to show the ability of both *A. areolatum* and a second species, *A. chailletii*, to penetrate nematode eggs and adult living females of three species of *Deladenus* nematodes.

CONTRIBUTED PAPERS Monday, 16:30-18:30

VIRUSES 2

Contributed paper. Monday, 16:30. **43**

Insect feeding induces transgenerational resistance to NPV in Lepidoptera

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When population density increases, insects experience a number of stresses as a result of crowding including alteration in food quality and quantity. These changes have been shown to alter the resistance of insects to pathogens. Previous studies have tended to investigate the impact of these factors individually: however, in nature density-related factors are likely to interact simultaneously. While changes that occur within a single generation have been well documented, we know less about the transgenerational impact of changes in food quality on disease resistance. We tested the impact and interaction of three factors that are likely to occur in insect populations when density rises using the western tent caterpillar, *Malacosoma californicum pluviale*. Western tent caterpillars exhibit population cycles every 8-11 years, which are characterized by NPV epizootics at high density. We manipulated food quality, food quantity and the presence of phylloplane bacteria in the parental generation and measured the impact on immunity and resistance to NPV in the offspring. The treatments, particularly the foliar treatments, had clear impacts on the disease resistance of the offspring generation: however, not necessarily in the direction predicted. We discuss these data in relation to how changing levels of susceptibility could influence population cycles in these forest insects.

Contributed paper. Monday, 16:45. **44**

The resistance of *Cydia pomonella* against baculoviruses is provoked by a mutation of the immediate-early *pe38* gene of *Cydia pomonell*a granulovirus

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The *Cydia pomonella* granulovirus (CpGV) (*Baculoviridae,* genus *Betabaculovirus*) is a worldwide used biological agent to control the infestation of pome fruits by codling moth (*C. pomonella* L.). In 2005, first CM field populations resistant to commercial CpGV products containing the isolate CpGV-M (so-called Mexican isolate) were discovered in Europe. These resistant CM populations showed 1,000 – 100,000fold reduced susceptibility to CpGV-M when compared to normally susceptible CM populations. Infection experiments with isolates from different geographical origins showed that various CpGV isolates were able to overcome CM resistance in the genetically homogenous resistant laboratory CM strain. Molecular analysis of these resistance overcoming isolates (-I12, -I07, -S, and -E2) showed that the only genomic difference, which all resistance overcoming isolates have in common, is a single common 24 nucleotide indel mutation coding for eight amino acids within the immediate-early gene *pe38*. Phylogenetic analyses presume that this mutation is an insertion within the genome of CpGV-M.
Therefore, the role of *pe38* in overcoming the resistance of CM was analyzed by constructing knockout and rescue pseudoviral mutants based on a CpGV-M bacmid. According to the source of *pe38*, we could show that the pseudoviruses are infective against susceptible larvae only - in the case of *pe38* from CpGV-M - or against both susceptible and resistant larvae - in the case of *pe38* from CpGV-S. Therefore, we conclude that *pe38* is not only an essential factor for the infectivity of CpGV but also the key factor in overcoming CpGV resistance in CM.

Contributed paper. Monday, 17:00. **45**

CpGV-R5 allows replication of CpGV-M in resistant host insect larvae

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In resistant codling moth larvae, CpGV-M replication is blocked at an early step in all tissues. Among others, the CpGV-R5 isolate is able to overcome this resistance. A genetically heterogeneous virus population, containing 1% CpGV-R5, and

99% CpGV-M has been used to infect non permissive host populations. Unexpectedly, this mixture, and the OBs recovered from killed larvae performed 100 times and 2000 times better than CpGV-M used alone, respectively. qPCR analysis using specific markers for each viral isolate was performed. The viral mixture CpGV-R5, 1%, and 99% of CpGV-M was amplified on permissive (Cp_{NPP}) and non-permissive (R_{GV}) populations and their offspring was tested for their respective proportion of each kind of marker. On permissive host, the R/M markers ratio raised to 15/85. On resistant host, a similar R/M ratio (12/88) was obtained, indicating that CpGV-M has been able to perform a complete replication cycle in a non-permissive host. These results suggest that in the presence of a small proportion of CpGV-R5, CpGV-M is able to replicate in resistant hosts. Accordingly, CpGV-R5 seems to act as a helper for CpGV-M genomes. Understanding the mechanism involved in the unlocking of the replication process opens the possibilities of innovative control strategies.

Contributed paper. Monday, 17:15. **46**

Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*

Agata K. Jakubowska[1]; Melania D´Angiolo[1]; Rosa M. González Martínez[1]; Anabel Millán Leiva[1]; Arkaitz Carballo[2]; Rosa Murillo[2]; Primitivo Caballero[2]; Salvador Herrero[1]
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Viral covert infections in invertebrates have been traditionally attributed to sublethal infections that did not reach enough viral titer to establish an acute infection. Recent studies are revealing that, although true for some viruses, other viruses may follow the strategy of establishing covert or persistent infections without producing the death of the host. In the last years, a large number of viruses causing covert infections in all type of hosts have been identified, mostly due to the revolution in the sequencing technologies. The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) is a worldwide pest that causes significant loses to agricultural and ornamental plant industries. A comprehensive transcriptome analysis of the larval stage of *S. exigua* revealed the presence of an important number of unigenes belonging to novel RNA viruses, most of them from the order *Picornavirale*s. In order to characterize *S. exigua* viral complex, we have completed the genomic sequences of three picorna-like viruses, two of them representing new members of the family *Iflaviridae* and a third one defining a new family. Additional studies have been performed to determine their morphology, infectivity, tissue distribution and abundance in the larval hosts. Influence of these viruses on the insect fitness as well as their effect on other viral and bacterial entomopathogens used for the control of this pest is also discussed.

Contributed paper. Monday, 17:30. **47**

Mixed SeMNPV genotypes comprised transmission capacities and insecticidal properties

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Recent studies have demonstrated that transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) parents to offspring (vertical transmission) is frequent and could contribute to biological control of this pest by causing viral mortality in the pest population in susccessive cropping cycles. The aim of this work was to study the efficacy of using mixtures of two SeMNPV genotypes that had either high insecticidal properties (SeG25) or the capability to be transmitted through host generations (SeAl1). Mixed populations containing 25 and 75% of SeG25 resulted in increased pathogenicity (LC_{50}) compared to the SeAl1 genotype. However in terms of virulence (mean time to death) and productivity (OBs/larva), no differences were observed between the individual genotypes or their mixtures. The capacity to induce persistent infections by each genotype and their mixtures was evaluated using qPCR (*DNA-polymerase* gene) in adult survivors of a sublethal dose of the virus. The prevalence of covert infection varied between 70 and 100% in adults that survived inoculation with the vertically transmitted genotpype Se-Al1. The adult survivors to the mixtures and the SeG25 genotype alone are currently being analyzed to determine covert infection. Finally, field trials were carried out to evaluate the capacity of mixed virus populations to establish covert infections in greenhouse conditions. Adults developed from larvae collected in experimental plots sprayed with either single genotypes or one of the mixtures 75%Al1+ 25%G25 (75:25) and 25%Al1 +75%G25 (25:75) are being processed currently. The F_1 offspring from adult survivors of SeAl1, 75:25, 25:75, SeG25 and control treatment did not showed differential susceptibility to a 25:75 mixture of OBs. The implications of these findings will be discussed.

Contributed paper. Monday, 17:45. **48-STU**

A novel mode of resistance of codling moth against *Cydia pomonella granulovirus*

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The codling moth (CM, *Cydia pomonella*) is one of the most devastating pests in nearly all pome fruit growing regions. An alternative to the application of chemical insecticides is the application of *Cydia pomonella granulovirus* (CpGV) (family *Baculoviridae*), which is registered as biological control agents in 34 countries worldwide. Since 2005, CM populations with a reduced susceptibility to CpGV products have been reported from about 40 plantations in seven European countries. For many of these CM populations, the resistance could be traced back to a single, dominant allele that is linked to the sex chromosome Z. CpGV-M, the so-called Mexican isolate, was the common agent used in all commercial CpGV products registered in Europe. Currently, resistance management strategies are based on the application of improved CpGV products, containing resistance-overcoming isolates. However, a CM field population, termed NRW-WE showed even resistance to most resistance overcoming CpGV isolates, suggesting a second mode of CpGV resistance.
In order to elucidate the inheritance of this type of resistance and after failure of single crossing experiments, successive mass crossings under virus pressure were carried out to establish a genetically homogenous resistant strain of the CM population NRW-WE. Subsequent reciprocal crossing experiments with the resulting CM strain and a susceptible laboratory CM strain (CpS) followed by bioassays fitted to a dominant but autosomal inheritance model. Further analyses of the mode of resistance are under way. .

Contributed paper. Monday, 18:00. **49**

The effects of temperature on *Cryptophlebia leucotreta* granulovirus (GrleGV-SA) in mortality rates of false codling moth larvae *Thaumatotibia leucotreta*

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False codling moth (FCM), *Thaumatotibia leucotreta* is a major citrus pest in South Africa. *Cryptophlebia leucotreta* granulovirus (GrleGV-SA) has been found to be a successful biological control agent for FCM. South Africa grows citrus in many different geographical areas throughout the country that experience different temperature differences; this in turn could affect the efficiency of the virus upon the larvae. The aim of this study was to determine the effectiveness of the virus on larvae at temperatures ranging between 15-35°C. Unpaired T-tests, one-way ANOVA tests and post-Hoc Tukey's HSD tests were conducted on both virus and control treatments to test for significant differences among different temperatures as well as between the virus and control treatments. The number of deaths between infected and control treatments were significantly different at all temperatures. The differences between treatment mortality times were significantly different for all infection stages except the final death stage (5^{th} stage). The virus was found to be most efficient at higher temperatures since the larvae grow faster at higher temperatures. The virus was found to have very little effect at 15°C. These results should assist with the control of FCM in citrus orchards, and in particular would affect the timing of applications, to ensure that the virus is used at its maximum efficiency.

Contributed paper. Monday, 18:15. **50**

Enhancement of insecticidal activity of a nucleopolyhedrovirus isolated from *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) by coinfection with granulovirus

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Spodoptera frugiperda is a polyphagous pest with wide geographical distribution. Biological control of this pest has included the use of its nucleopolyhedrovirus SfMNPV, which has shown high potential as biopesticide with efficacies higher than 80% but with some disadvantages related with cost production and time of action. In this sense, other viruses as betabaculovirus (GV) may act as synergists, increasing the insecticidal activity of NPVs. In this work, a Colombian granulovirus isolated from *S. frugiperda* larvae (VG008) was mixed with two different NPVs samples, one corresponding to a wild virus NPV003 and other corresponding to a pure genotype variant obtained from NPV003 (NPV003-A). Each mixture was evaluated in different proportions and in five different concentrations since 1×10^4 OB/mL to 1×10^8 OB/mL. For each mixture, the median lethal concentration (LC_{50}) and mean time of mortality (MTM) were determined by laboratory bioassay in second instar larvae of *S. frugiperda.* Majority of mixtures between the VG008 and NPV003 showed a higher biological activity compared with each individual isolate, confirming the coinfection enhancement effect. The mixture corresponding to 2.5% of VG008 and 97.5% of NPV003, showed the highest enhancement of the NPV insecticidal activity with a decrease of 9.92 times in the LC_{50} and 4 days (96 hours) in the MTM. This virus mixture was selected and will be used as an active ingredient for the development of a new biopesticide based on both viruses in order to improve NPV efficacy for controlling the pest in the field.

FUNGI 2

Contributed paper. Monday, 16:30. **51**

Rapid and simple method for overnight development of strain-specific markers: A case study with the commercial *Beauveria bassiana* strain, GHA.

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Genetic markers have proved useful for assessing taxonomy and identifying specific-strains of entomopathogenic fungi. We targeted *Beauveria bassiana* commercial strain, GHA to develop a new reliable, simple, specific, sensitive and cost effective method that allows specific detection and discrimination of GHA from other *Beauveria* strains. We applied a combination of software with intrinsic manipulations to design GHA strain-specific primers by exploiting available *Bloc* nuclear intergenic sequences of GHA and other *Beauveria* strains. The generated primers were used in PCR assays to probe strains of *B. bassiana* (50), *Beauveria pseudobassiana* (13), *Beauveria bronginiartii* (3), *Beauveria amorpha* (2), *Beauveria vermiconia* (2), *Beauveria asiatica*, *Beauveria australis*, *Beauveria kipukae*, *Beauveria malawiensis*, *Beauveria sungii* and *Beauveria varroae*. In the specificity test, we amplified the expected target gene and ~ 300-bp-fragment from *B. bassiana*, GHA DNA. All other tested strains/isolates reacted negatively with the exception of four out of fifty *B. bassiana* strains that produced positive signals. In addition, the designed primers were highly sensitive; capable of detecting ~20 pg/µl of GHA genomic DNA. For operational feasibility, the newly designed marker would be used for studying the ecology, persistence and monitoring autodissemination of post-released GHA in the environment. To date, our methodology and associate protocol could be considered the simplest with high sensitivity and specificity, and most cost effective strategy for strain-specific marker design in the highly heterogeneous *Beauveria* species complex. Our approach provides a general framework that can be readily or easily adapted for designing strain-specific markers targeting any organism of choice.

Contributed paper. Monday, 16:45. **52-STU**

The functions of two Cu/Zn-superoxide dismutases and a Fe-superoxide dismutase in regulating the growth, antioxidation, UV tolerance and virulence of *Beauveria bassiana*

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The biocontrol potential of filamentous entomopathogenic fungi, such as *Beauveria bassiana*, depends not only on the virulence of a candidate strain to target pests but also on its tolerance to high temperature and solar UV irradiation often encountered in the field. The stress of UV, heat, drought, or

chemical may induce the production of cellular reactive oxygen species (ROS), which cause damages to most biomolecules such as DNA, protein, and lipids. Fungal superoxide dismutases (SODs) that detoxify superoxide anions could be putative virulence factors for entomo-pathogenic fungi. Three genes encoding SODs have been identified in the *Beauveria bassiana*: a cytoplasmic Cu/ZnSOD (BbSod1), a mitochondrial FeSOD (BbSod4) and a cell-wall Cu/ZnSOD (BbSod5). During growth, *BbSod4* was weakly expressed compared with other SODs and the deletion of *BbSod4* was lethal. To probe their effects on the biocontrol potential of *B. bassiana,* Δ*BbSod1*, Δ*BbSod5* and three hairpin RNA-interfered (RNAi) mutants were constructed and assayed for various phenotypic parameters in conjunction with Δ*BbSod1*/ *BbSod1*, Δ*BbSod5*/ *BbSod5* and wild-type (control strains). The knockout mutants showed phenotypic alterations, including delayed sporulation and impaired conidial quality, but little change in RNAi mutants. Their mycelia or conidia became more sensitive to menadione or H_2O_2 induced oxidative stress but had little change in resistance to hyperosmolarity and wet-heat stress. Their UV tolerance and virulence was also impaired. Transcriptional changes of five *Sod* genes and other relative genes described try to explain the phenotypic changes among the mutants. Our finding highlight that these three Sods regulate the oxidative resistance in different method, thereby exerting profound effects on the fungal biocontrol potential.

Contributed paper. Monday, 17:00. **53-STU**

Effect of temperature, water activity and UV-B radiation on conidia germination and colony growth of *Beauveria bassiana* isolates from soil and phylloplane

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Have entomopathogenic fungi phylloplane isolates any advantage over soil isolates in terms of environmental competence and virulence?. To address this question, 20 *Beauveria bassiana* isolates from soil and phylloplane of two holm oak ecosystems in Southern Spain pathogenic to medfly *Ceratitis capitata* adults and belonging to different type sequences and genotypes as interfered from EF-1α, Bloc and microsatellites were selected and their comparative response to temperature, water activity and UV.-B investigated. Effect of temperature on germination and colony growth rate was monitored in the range of 15-35ºC, with optimum temperature ranging from 23.8-28.7 ºC. All isolates showed maximum germination values between 1 and 0.996 water activity (a_w). Germination at a_w values lower than 0.928 were not observed for any isolate. Moreover, conidia were exposed to different irradiances (920 and 1200 mWm^{-2}) during 2, 4 and 6 hours, and germination, culturability and mycelia growth were evaluated. These results show that a "recovery" of the fungal propagules could occur after being exposed to UV-B, even if such recovery is lower for longer exposure times (6h) and irradiance (1200 mWm^{-2}). Therefore, the answer may be now addressed: the fungus isolation habitat does not always provide advantage in terms of environmental competence.

Contributed paper. Monday, 17:15. **54**

Non-target aquatic arthropods testing of *Metarhizium* strains and their crude extracts produced by solvent extraction and nanofiltration technology

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Strains of insect pathogenic fungi within the genus *Metarhizium* have increasingly been developed for the control of pest species. Risk assessment studies are a prerequisite before the fungus can be registered as a plant protection product. In this work we determined the risks posed by preparations of secreted metabolites and viable conidia to two aquatic, ecological toxicity indicator species, *Artemia salina* and *Daphnia pulex.* Organic solvents (OS) are normally used to extract the metabolites but they pose a risk to human health and the environment. Nanofiltration (NF) is an environmentally responsible technology that can be used to extract the metabolites as an alternative to the OS. Since risk assessment of each secondary metabolite produced by EPF could be a long and expensive process, the RAFBCA-REBECA decision scheme proposes evaluation of the risks posed by crude extracts. Therefore, three fungal strains (BIPESCO5, ARSEF 4556, and ARSEF 3297) were produced in three different culture media [Czapek-dox + peptone, Czapek-dox + yeast, and 10:1 (C:N ratio)], and their metabolites extracted by OS and NF methods. The chromatographic profiling of all the products was determined and their toxicity tested against *A. salina* and *D. pulex.* Concomitantly, the pathogenicity of the strains was tested against these non-target arthropods. At a relatively high dose (10^8 conidia ml^{-1}), the conidia could cause 69% and 75% mortality in *A. salina* and *D. pulex* respectively. Both arthropods were sensitive to metabolites. Mortality depended on the fungal strain, extraction method, and test organism. Our study showed that *A. salina* and *D pulex* mortality was due to the combination of *Metarhizium* conidia induced stress as well as secreted metabolites.

Contributed paper. Monday, 17:30. **56-STU**

Development of analytical methods for the analysis of *Metarhizium brunneum* metabolites in crop matrices

Judith Taibon[1,2], Sonja Sturm[1], Christoph Seger[1,3], Hermann Stuppner[1], Hermann Strasser[2]
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The main secondary metabolites produced by the entomopathogenic fungus *Metarhizium brunneum* are destruxins (dtxs), cyclic hexadepsipeptides, which exhibit a wide variety of biological activities. Overall they are best known for their insecticidal and phytotoxic activities. Since the fungus is used for biological control of insect pests there are some concerns regarding whether the produced secondary metabolites entail risks to humans and the environment. To asses if the major secondary metabolites secreted by *M. brunneum* enter the food chain a two-step sample preparation protocol, consisting of the sample extraction by the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method followed by the sample purification by offline solid phase extraction on a reversed phase material was established. For the analysis and quantification of dtx congeners a fast and selective UHPLC-DAD/TOF-MS method based on a previously developed method was optimized. It

turned out that the QuEChERS-method is an efficient way to extract dtxs from different crop matrices. Using offline SPE for the clean-up of the samples analytes can be separated from disturbing matrix compounds and quantified by the UHPLC-DAD/TOF-MS method.

Contributed paper. Monday, 17:45. **57-STU**

α-1, 2-mannosyltransferase ktr1, ktr4 and kre2 regulate positively growth, conidiation, viability, virulence, and multi-stress tolerances in *Beauveria bassiana*

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Entomopathogenic fungus *Beauveria bassiana* is a mycoinsecticide against arthropod pests. Three α-1, 2-mannosyltransferase proteins (Ktrp) named Bbktr1, Bbktr4 and Bbkre2 are responsible for extension of the second and third mannose residues on secretory protein. Here, we characterized the role of three Ktrp in *B. bassiana* and found that they were positive, but differential, regulators of the growth, conidiation, multi-stress tolerance and virulence of the entomopathogenic fungus. The three disruptions accompanied with their corresponding complement Δ*Bbktr1/ktr1*, Δ*Bbktr4/ktr4*, Δ*Bbkre2/kre2* and wild-type were constructed. Δ*Bbktr4 and* Δ*Bbkre2* grew 50–83% slower on nutrition-rich and limited media while Δ*Bbktr1* show similar colony sizes on all the tested media. Their conidial yields on a standard medium were reduced by 31–96%, accompanied with abnormal germination. All the mutants became significantly less tolerant to most stresses of cell wall perturbation, high osmolarity, oxidation, wet heat and UV-B irradiation during colony growth. Furthuremore, the Ktrp mutants were altered in cell wall structure and composition, which contributed to the thickness of cell wall, increased sensitivity to lyase, the low conidial hydrophobicity and cell surface carbohydrate epitopes. Coincidentally, the attenuated cell wall in Ktrp mutants also brought out the more protoplast to release. Remarkably, insect bioassays revealed decreased virulence in Δ*Bbktr4*, Δ*Bbkre2* for 18% and 1.2-fold with topical application, and 31% and 26% with intrahemoceol injection. Our findings revealed that Ktrp plays a central regulatory role in *B. bassiana*.

TUESDAY - 5 August

SYMPOSIUM 3 (Fungi) Tuesday, 8:00-10:00

Fatal attraction: Fungi and Odours in Deadly Combinations for Pest Control

Symposium. Tuesday, 8:00. **58**

Conifer - bark beetle - fungus interactions

Tao Zhao[1], Paal Krokene[2], Anna-Karin Borg-Karlson[1]
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Tree-killing bark beetles such as the spruce bark beetle (*Ips typographus*) have huge economic and ecological impacts in conifer forests worldwide. Just in the last 25 years the spruce bark beetle has killed millions of cubic meters of Norway spruce (*Picea abies*) in Europe. Trees are killed by a combination of pheromone-mediated mass-attacks and infection with phytopathogenic bluestain fungi vectored by the beetles. *Ceratocystis polonica,* the most virulent fungal associate of the spruce bark beetle, can kill healthy trees in the absence of beetle attack if it is experimentally inoculated into the bark at high densities. Norway spruce protects itself against combined beetle-fungus attacks by multiple preformed and inducible defense mechanisms. Structurally diverse mixtures of mono-, sesqui- and diterpenes are central components of these defenses. Preformed terpenes stored in resin ducts in the bark and sapwood may repel or inhibit initial attacks. Terpene levels increase tremendously following induction by e.g. fungal infection or application of methyl jasmonate (a defense-inducing plant hormone). This induced terpene response reduces pheromone emission by the spruce bark beetle and inhibits tree colonization in a dose-dependent manner. However, fungal associates of the spruce bark beetle can greatly reduce monoterpene levels in the tree by biotransforming them to oxygenated monoterpenes. In addition, the fungi also produce different metabolites which may play multiple roles in bark beetle host finding and colonization. These observations demonstrate the complicated interactions between conifer-bark beetle-fungi.

Symposium. Tuesday, 8:20. **59**

Carbon dioxide as an orientation cue for western corn rootworm and wireworm larvae - implications for an attract and kill approach using entomopathogenic fungi

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The larvae of soil dwelling insects use carbon dioxide gradients, established by growing roots, to orientate towards their host plants. This long distance orientation cue is complemented by other volatile cues to finally accept a host plant for feeding. Previous application strategies using entomopathogenic fungi for soil pest control were using high concentrations of spores per m^2, set against competing microorganisms in the rhizosphere. In the attract and kill approach the strategy is turned upside down: larvae voluntarily make their way to the spores, contained in capsules emitting CO_2. When near to these capsules, probability of larval infestation with spores is higher. However, to make this strategy work, the capsules need to fulfill several prerequisites, such as building up a gradient significantly higher than the background CO_2 concentration in the soil, maintained for at least several weeks, and the larvae need to be attracted to the capsules to feed on them. In lab experiments we assessed the larval behavior of corn rootworms and wireworms towards these artificial CO_2-capsules. Both pest larvae were attracted by the capsules, but only stayed for short periods at these sites. Thus, additional compounds need to be incorporated into these capsules to increase their attractiveness for the larvae. In German field experiments these capsules, combined with *M. brunneum*, were used in potato fields for wireworm control. Treatments resulted in significantly lower tuber damage in some, but not all fields. Necessary improvements of the attract and kill strategy for anapplication in the field are discussed.

Symposium. Tuesday, 8:40. **60**

Different behavioral responses in specialist and generalist natural enemy interactions (predators and fungi) in a strawberry-mite pest system

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Natural enemies like arthropods and entomopathogenic fungi both contribute to the natural regulation of pests in many crops. As arthropod natural enemies have evolved, they have become a part of a complex multitrophic system and they exist alongside species of entomopathogenic fungi. Some of these entomopathogenic fungi may actually also be a potential threat to arthropod natural enemies. Both arthropod predators and entomopathogenic fungi are important biological control agents of the two spotted spider mite, *Tetranychus urticae* in strawberry. Previous studies on the interactions between these two types of natural enemies show variable results in regards to synergistic/antagonistic effects. We speculated if the degree of specialization of the predator or the fungus could play a significant role. Therefor a behavioral study was conducted to investigate the searching and feeding time of predators (two species tested) in the presence of entomopathogenic fungal spores (two species tested). The predator species used in this study were the generalist predatory bug, *Orius majusculus* and the specialist predatory mite, *Phytoseiulus persimilis*. The entomopathogenic fungal species used was the generalist *Metarhizium brunneum* and the specialist *Neozygites floridana*. Predator behavior was recorded by observations in an experimental setup where the predator was given a choice between two strawberry leaf discs; one with entomopathogenic fungal spores and one without, and both with healthy *T. urticae*. Results suggest that searching and feeding times of both predator species was lower on leaf discs with presence of *M. brunneum* spores compared to no fungal spores. On leaf discs with *N. floridana* spores the searching time of both predators was higher compared to no fungal spores. *O. majusculus* spent more time feeding on prey on the leaf disc with spores of *N. floridana* than on leaf discs without spores, while *P. persimilis* spent less time feeding on the leaf discs with *N. floridana* spores, compared to leaf discs with no fungal spores. Results indicate that the degree of specialization of the beneficial organisms plays a role in the interaction between arthropods and entomopathogenic fungi. Such interactions are important to consider when biological control using several biological control agents is developed.

Symposium. Tuesday, 9:00. **61-STU**

How *Fusarium graminearum* influences insect-plant interactions

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Fusarium Head Blight (FHB) disease affects cereals globally, and is caused by a range of toxigenic fungi from the genus *Fusarium*. Wheat (*Triticum aestivum*) is most susceptible to FHB during flowering. The role of insect pests in FHB epidemiology is poorly understood, so the objective of this work was to determine the interactions between the most dominant FHB pathogen, *Fusarium graminearum*, and insect pests that would co-localise on host plants. Grain aphids, *Sitobion avenae*, were used as they are known to colonise wheat ears during flowering. Wheat ears were treated with combinations of fungal inoculum and grain aphids transferred from either healthy or infected previous hosts. Ears treated simultaneously with *F. graminearum* inoculum and aphids incurred significantly higher disease severity, pathogen DNA and accumulation of the mycotoxin deoxynivalenol than ears treated with *F. graminearum* inoculum alone. Olfactometer assays using headspace samples of volatiles from wheat ears inoculated with the pathogen showed that *F. graminearum*-induced volatiles were repellent to aphids. Chemicals responsible for repellency were identified via GC-linked electroantennography and GC-MS followed by olfactometer assays of the electrophysiologically active components. Furthermore, decreased fecundity and survival was observed for aphids fed with *F. graminearum* symptomatic ears. Aphid feeding increased disease progression, therefore benefitted the colonising pathogen, possibly by altering plant defence responses. However, disease induction negatively impacted on aphid survival and reproductive success. Exhibition of a repellent response by aphids to volatiles from diseased plants can be interpreted as an adaptation by aphids to evade the inhospitable enviroment created by the pathogen.

Symposium. Tuesday, 9:20. **62**

Plant-microorganism interactions that shape host-plant selection in the grapevine moth

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Plant-micro-organisms associations may play a role in shaping plant-herbivore interactions. Here, we tested whether the inoculation of a host-plant with a variety of microorganisms would be able to affect the attraction to the plant, the oviposition preference and the fitness of an herbivorous insect. We worked on the system of a phytophagous species (grapevine moth *Lobesia botrana*), its host plant (grapevine *Vitis vinifera*) and the microorganisms associated with the plant. In vineyards, *L. botrana* use a volatile signal to locate the host-plant from a distance and to oviposit on grape. In our experiments, the attraction from a distance and the oviposition preference of the moth were influenced by the microbial activity on the plant. In addition, the quality of the host plant as larval food was importantly changed by the presence of pathogenic or opportunistic microorganisms on the plant. Taken together our results indicated a major role of endemic microorganisms on *L. botrana* host-selection and life-traits. Microbial volatiles appear to be a major cue mediating this kind of interaction.

Symposium. Tuesday, 9:40. **63**

Effect of host plant on aphid susceptibility to the fungal pathogen *Pandora neoaphidis.*

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Virulence of the aphid-specific fungus *Pandora neoaphidis*, as

measured in dose-response assays, was compared against the pea aphid, *Acyrthosiphon pisum*, that had been reared on different host plant species. *A. pisum* were reared on dwarf bean then inoculated with *P. neoaphidis* and returned to dwarf bean or inoculated and transferred to field bean, pea or lucerne. The smallest estimated median lethal concentration (LC_{50}) was 7.7 conidia mm^{-2} for aphids returned to dwarf bean, with LC_{50}s of 13.0 and 14.6 conidia mm^{-2} for aphids transferred to field bean or pea, respectively. The largest LC_{50} was achieved when aphids were transferred to lucerne: 2941.0 conidia mm^{-2}. In a subsequent experiment, *A. pisum* were reared on either pea or dwarf bean for four generations before bioassays. The LC_{50} for aphids reared and incubated on dwarf bean was 7.3 conidia mm^{-2}, compared to 13.3 and 15.3 conidia mm^{-2} when aphids were transferred between dwarf bean and pea, or pea and dwarf bean, respectively. The LC_{50} for aphids reared then incubated on pea plants was 27.9 conidia mm^{-2}. Overall, the virulence of *P. neoaphidis* was greatest when *A. pisum* was reared and maintained on dwarf bean, the plant used for long-term routine culturing of the aphid. In conclusion, virulence of *P. neoaphidis* was influenced by host plant and particularly by the plant species to which the host aphid had become adapted. Plant resources may affect the population dynamics of *P. neoaphidis* and could result in a greater impact on aphid herbivores that are not suffering physiological stress related to a change in host plant.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

NEMATODES 2

Contributed paper. Tuesday, 8:00. **64**

Entomopathogenic nematode behavioral responses to chemical cues from cadavers.

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Entomopathogenic nematodes (EPN) are exposed to a range of cues in the soil. To the extent these cues are positively associated with the presence of insect hosts, one might hypothesize that EPN would respond positively to such cues. Decomposing animals release many different chemical compounds into the soil, attract large numbers of foraging insects, and produce large numbers of insect larvae. Thus, these chemical compounds may serve as an important cue for foraging EPN. We hypothesized the *Steinernema feltiae* and *Steinernema glaseri* IJs would respond generally positively to two particular compounds (putrescine and cadaverine) produced during animal cadaver decomposition. We further hypothesized that *S. feltiae* would respond more strongly to putrescine, and that *S. glaseri* would respond more strongly to cadaverine. We initially used standard agar-based "bulls-eye" attraction assays, and assessed *S. feltiae* and *S. glaseri* responses to diffusion discs soaked in 5 µl of 50, 100, 500, and 1000 µmol concentrations of each of the two compounds. We followed those agar trials with more realistic small sand column assays, assessing responses to the compounds when they were presented with additional stimuli such as host presence. On agar, responses differed between the different EPN species, chemical compounds, and concentrations, but the chemicals were never attractive and often strongly repellent. Responses were more complex in the sand columns; in particular, the compounds seem to attract more IJs to areas that also contained hosts.

Contributed paper. Tuesday, 8:15. **65**

The *Wolbachia* Endosymbiont as a Nematode Drug Target for Control of Human Filariasis, a Neglected Tropical Disease and Other insect Borne Pathogens

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Most human filarial nematode parasites and arthropods are hosts for a bacterial endosymbiont, *Wolbachia*. In filaria, *Wolbachia* are required for normal development, fertility and survival, whereas in arthropods, they are largely parasitic and can influence development and reproduction, but are generally not required for host survival. Due to their obligate nature in filarial parasites, *Wolbachia* have been a target for drug discovery initiatives using several approaches including diversity and focused library screening and genomic sequence analysis. *In vitro* and *in vivo* anti-*Wolbachia* antibiotic treatments have been shown to have adulticidal activity, a long sought goal of filarial parasite drug discovery. In mosquitoes, it has been shown that the presence of *Wolbachia* can inhibit the replication of certain viruses, such as Dengue, Chikungunya, Yellow Fever West Nile, and the infectivity of the malaria-causing protozoan, *Plasmodium* and filarial nematodes. Furthermore, *Wolbachia* can cause a form of conditional sterility that can be used to suppress populations of mosquitoes and additional medically important insects. Thus *Wolbachia*, a pandemic endosymbiont offers great potential for elimination of a wide-variety of devastating human diseases.

Contributed paper. Tuesday, 8:30. **66**

Differential PirAB expression of the entomopathogenic bacterium *Photorhabdus luminescens* (Enterobacteriaceae) based on tissue association and portal of entry to the insect host

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Photorhabdus bacteria gain access to an insect host by their association with the free-living infective juvenile stage (IJ) of *Heterorhabditis* nematodes. Penetration of the insect can be achieved through three different portals of entry: a) digestive (mouth, anus), b) tracheal (spiracles) and c) integument. Studies have shown that *Photorhabdus* may colonize other tissues before they establish in the insect's hemocoel, the final destination for full release of bacterial symbionts and completion of their life cycle. It is likely that *Photorhabdus* employs effectors related to virulence factors in pathogens for adhesion, invasion, and intracellular growth in its host's cells. In this study we investigated tissue aggregations and virulence factors by measuring *Pir*AB toxin expression of *Photorhabdus luminescens* (TT01) in different insect tissues and concurrent to different portals of entry used by their nematode hosts.

Contributed paper. Tuesday, 8:45. **67 STU**

Candidate Virulence Loci in Pan-Genome of the Entomopathogenic Bacterium, *Xenorhabdus bovienii* (Gamma-Proteobacteria: Enterobacteriaceae)

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Xenorhabdus spp. has dual life styles: they are pathogenic to insects and mutualistic with *Steinernema* nematodes. The nematodes vector the bacteria from one insect to another. In return, bacteria provide a suitable environment in the insect cadaver for the nematodes to maturate and reproduce. Each *Steinernema* spp. carries one *Xenorhabdus* sp. Contrarily, a *Xenorhabdus* spp. may associate with more than one nematode host. The most promiscuous bacterium is *X. bovienii*, which associates with nine *Steinernema* spp. In this study, we performed a comparative genomic analysis of nine *X. bovienii* strains to depict novel virulence factors. Furthermore, virulence assays were performed considering three different lepidopteran hosts. Results revealed that four *X. bovienii* strains were attenuated, whereas the other five were virulent. The genomic platform MicroScope was used to identify known and candidate genes that contribute to their pathogenicity. Additionally, loci involved in their association with the nematodes were investigated. Two loci were identified as novel candidates involved in the bacterium's ability to interact with both nematode and insect hosts. The first region appears to be specific to interactions with nematode partners. The second region contains a type six secretion system (T6SS), which is known to contribute to bacterial pathogenicity. We hypothesize T6SS may contribute to the bacterium's ability to cause death in a wide range of insect hosts. Further molecular studies are undergoing to expand our understanding on the role of these loci and their mode of action in the dual lifestyle of this bacterium.

Contributed paper. Tuesday, 9:00. **69**

Molecular mechanism of the nematicidal activity of *Photorhabdus luminescens* LN2 against *Heterorhabditis bacteriophora* H06 nematodes

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Photorhabdus luminescens subsp. akhurstii LN2 (Enterobacteriaceae) is a symbiont of entomopathogenic nematodes *Heterorhabditis indica* LN2 and showed nematicidal activity against *H. bacteriophora* H06 infective juveniles (IJs). The LN2 bacteria may secrete unidentified toxic factors lethal for the H06 nematodes. The trans-specific nematicidal activity of the bacteria against the non-symbiotic nematode may have an impact on competitive interactions when one insect host is co-infected by different nematode species. To explore the molecular mechanism of the trans-specific nematicidal activity of *P. luminescens* LN2 against *H. bacteriophora* H06, the complete genome of *P. luminescens* LN2 was sequenced; two mutagenesis libraries of *P. luminescens* LN2 were constructed using Tn5 transposon and rifampicin antibiotic respectively; the mutants from the libraries were tested for nematicidal activity and mutants negative for nematicidal activity were genetically and proteomically characterized. At least 9 putative proteins including DsbA, HlpA, RhlE, RplC, RpoB, NamA, NamB (a protein from T3SS), and 2 hypothetical proteins (similar to unknown protein YgdH and YggE of *Escherichia coli* respectively) were involved in the nematicidal activity of LN2 bacteria against H06 nematodes. This hypothesis was further confirmed by creating insertion-deletion mutants of corresponding genes. It seems that a big network system is involved in this nematicidal activity.

Contributed paper. Tuesday, 9:15. **70**

Natural products from entomopathogenic bacteria: Understanding the interaction of bacteria, insects and nematodes

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Entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* live in symbiosis with nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively, and together they are able to infect and kill several different insect larvae. We have shown recently by chemical analysis and genome sequencing that these bacteria are able to produce a huge variety of different low molecular weight natural products. These compounds show insecticidal but also antibiotic and anticancer activity and novel bacterial signalling compounds have also been identified.
Recent work indicates that several of the bacterial natural products are addressing different parts of the insect immune system in order to make sure that the bacteria can evade it and kill the insect host. As the nematode immune system shows the same basic principles, it is of high interest how the natural products can differentiate between insect prey and nematode host. We will present our recent finding on natural products and their natural targets as well as ways to improve the production of these – probably also pharmaceutically useful – compounds.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

VIRUSES 3

Contributed paper. Tuesday, 8:00. **71**

Characterization and formulation of a Colombian isolate of *Erinnyis ello* granulovirus (L.) (Lepidoptera: Sphingidae)

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Erinnyis ello (L.) is a polyphagous lepidopteran pest that may cause serious annual losses in the rubber industry. The use of granulovirus represents an interesting alternative as a biological control agent for this insect. One Colombian granulovirus isolate (VG010) was obtained from *E. ello* larvae in the field and was characterized at morphological, biological and molecular level. Occlusion bodies showed an oval morphology with a unique nucleocapsid, and a size of 302.9 ± 22 x 181.5 ± 16 nm. The VG010 viral genome size was estimated to be approximately 88.7 kb. Phylogenetic relationships based on selected gene sequences *lef-8, lef-9* and *gran* showed a close relationship between VG010 and another isolate from *E. ello* previously reported (M34-4), suggesting that these isolates are genotypic variants of the same viral specie. The mean lethal dose of VG010 against second instar *E. ello* larvae was 4.3×10^3 OBs/mL and the viral productivity ranged between 2.1×10^9 and 3.8×10^9 OBs/g of larval tissue. With this virus, a wettable powder formulation was

developed which photostabilized viral OBs against UVB radiation and improved shelf life. This product presented an efficacy of 99% for controlling the pest in laboratory and quality control limits for the product were established. This biopesticide constitutes a new tool with high quality and efficacy that needs to be scaled up and evaluated under field conditions in order to confirm its potential for controlling this important pest in rubber crops.

Contributed paper. Tuesday, 8:15. **72**

Production of the *Cydia pomonella* granulovirus (CpGV) in a heterologous host

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The codling moth (CM), *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), is considered one of the most significant pests of apples and pears in the Western Cape, South Africa. Traditionally, control measures have relied heavily on the use of broad spectrum insecticides. *Cydia pomonella* granulovirus (CpGV) has proved to be an effective alternative to chemical application. The main objectives of this study were to identify a novel South African isolate of CpGV and to ascertain the viability and shortcomings of producing CpGV in the heterologous host, false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). Initially four field collected isolates were compared genetically to two commercially available products. PCR amplification and sequencing of CpGV *granulin* and *egt* genes as well as single restriction endonuclease digestion of genomic DNA isolated from purified occlusion bodies indicated that the South African isolates were genetically similar to the Mexican strain. A further two isolates have been collected from the Langkloof (Eastern Cape) and Harrismith (Free State) areas in which there is no previous record of commercial virus application. Genetic comparisons are currently being conducted. Initial results indicate genetic variation in the Harrismith isolate when compared to the Mexican strain. Rearing parameters for CM and FCM, including fecundity, percentage hatch, larval developmental times and percentage mortality, were compared. The quantity of CpGV per larval unit was calculated for both FCM and CM. Mortality and virus yields were assessed by inoculating early 4th and 5th instar larvae with eight concentrations of purified CpGV. The mortality data obtained from the virus yield trials were used to establish the concentrations required to conduct surface dose bioassays against both FCM larval larvae. Dose and time response values for 4th and 5th instar FCM larvae were determined and used in establishing a virus production technique. Effective quality control parameters have been established to ensure the integrity of virus being produced, namely bioassay, RE analysis using *Hind* III as there is no recognition site for this enzyme in CpGV DNA and, lastly, development of a set of standards for a qPCR reaction, which can be used to calculate the proportion of CpGV in a mixed virus solution. If this production technique was to be successfully implemented into a mass production programme the cost of producing CpGV could be significantly reduced.

Contributed paper. Tuesday, 8:30. **73**

Post-translational cleavage of P74 of the Helicoverpa armigera single nucleopolyhedrovirus facilitates *per os* infection

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Baculovirus oral infection is mediated by binding and fusing of occlusion derived virus (ODV) with the microvilli of midgut epithelium under alkaline condition. Previous studies showed that ODV attachment protein, P74, of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) undergoes two sequential cleavage events, the primary one being conducted by the endogenous alkaline protease at an unidentified site and the secondary one by host midgut trypsin at amino acids R195/R196/R199. Here we report that Helicoverpa armigera single nucleopolyhedrovius (HearNPV) P74 was first cleaved after translation in the host cell and was not dependent on the endogenous protease during ODVs release. The cleavage produces two subunits which were not associated by disulfide bonding. Judging from the molecular mass of the subunits, the cleavage was predicted at an arginine and lysine (R/K) rich region in the middle of HearNPV P74. A series of site-directed mutants in this region were generated. Feeding experiments showed that the single or multiple mutations significantly impaired *per os* infectivity and mutagenesis of R334Q/R339Q/R344Q/R347Q eliminated the specific cleavage of HearNPV P74. A mutant of the proposed second cleavage site R220Q/R221Q/R224Q was also generated and bioassays showed that the region was essential for oral infection. The results suggested that although there are some differences during the first cleavage, P74 of both AcMNPV and HearNPV undergo two steps cleavage, and the cleavage sites are likely to be conserved in the two viruses. An integrated model of P74 cleavage is provided which sheds lights on the molecular mechanism of ODV entry.

Contributed paper. Tuesday, 8:45. **74 STU**

Isolation, genetic characterisation and evaluation of biological activity of a novel South African *Phthorimaea operculella* granulovirus (*Phop*GV)

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The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a major pest of solanaceous crops in sub-tropical and tropical regions worldwide. This pest has developed resistance to many traditional pesticides, thus alternate means of control are required to protect the R2.5 billion (€168 million) potato industry in South Africa. The *Phthorimaea operculella* granulovirus (PhopGV) is considered a promising biopesticide that can be incorporated into integrated pest management programmes. Several PhopGV isolates recovered from geographically different insect populations have been genetically characterised and the full

genome of the Tunisian PhopGV-1346 isolate has been sequenced, providing a reference strain for comparison with novel isolates. This study reports the identification and genetic characterisation of a South African PhopGV isolate recovered from a *P. operculella* colony reared in the laboratory. Sequencing of the *lef*-8, granulin and *egt* genes confirmed the identity of the virus as PhopGV. Phylogenetic analysis of egt sequences grouped PhopGV-SA together with the Kenyan and South American isolates. Virulence evaluation against *P. operculella* larvae using surface dose and egg dip methods are currently underway and the preliminary data indicate that the virus has potential for development as a biopesticide for control of the pest in both the field and storage.

Contributed paper. Tuesday, 9:00. **75**

Genetic and biological characterisation of a novel South African *Plutella xylostella* granulovirus, PlxyGV-SA

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The diamondback moth, *Plutella xylostella* (L.) (Lep, Plutellidae), is a serious world-wide pest of cruciferous crops, with a global estimated cost of control and damage amounting to approximately US$4–5 billion annually. The *P. xylostella* granulovirus (PlxyGV) is considered a promising alternative to synthetic chemical insecticides and *Bt* insecticidal proteins for control due to the development of resistance in pest populations. Several PlxyGV isolates have been genetically and biologically characterised although many of these have not been commercialised as bio-pesticides. This is the first study to describe a novel South African PlxyGV in terms of genotype and biological activity. PlxyGV was recovered from an overcrowded laboratory *P. xylostella* colony established using field-collected insects. Occlusion bodies (OBs) were extracted from diseased larvae and purified by glycerol gradient centrifugation. PlxyGV-SA was genetically characterised by restriction endonuclease (REN) analysis of genomic DNA, and PCR amplification and sequencing of *granulin, ecdysteroid UDP-glucosyltransferase (egt), late expression factor 8 (lef-8) and late expression factor 9 (lef-9)* genes. Comparison of PlxyGV-SA REN profiles with those of PlxyGV-Japan (GenBank accession No. AF 270937.1) and other documented PlxyGV isolates together with sequence and alignment data showed that PlxyGV-SA is genetically unique. Neonate larvae were more susceptible to PlxyGV-SA infection than fourth instars at the same virus concentration. Biological activity determined by surface dose bioassays was estimated to be 3.56×10^5 OBs/ml (LC_{50}), which is comparable with values obtained in similar studies. These results suggest that PlxyGV-SA has significant potential for development as an effective biopesticide for the control of *P. xylostella* in the field.

Contributed paper. Tuesday, 9:15. **76-STU**

Comparative transcriptome analysis of CpGV-M in susceptible and resistant codling moth Cydia pomonella

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The *Cydia pomonella* granulovirus (CpGV) is commercially widely used and a cornerstone in the control of codling moth, *C. pomonella* L. (CM), in both organic and integrated pome fruit production. Recently, nearly 40 CM populations resistant to products based on the Mexican isolate CpGV-M have been located in Europe. So far, new CpGV isolates overcoming this resistance were identified and are applied in orchards with resistant CM populations. However, only limited information on the infection process of CpGV is available. To gain a better understanding of the interaction between CpGV-M and its host microarray analyses of the transcription of CpGV-M genes in the midgut of susceptible and resistant CM individuals was performed. Therefore, CM larvae were infected with CpGV-M and RNA samples were taken from midguts between 0 and 120 h post infection. Microarray analysis of the susceptible CM strain resulted in a detailed overview of the temporal transcription of all 143 CpGV-M genes. Four representative gene clusters were identified by performing a k-means clustering. Some correlation between the promoter motif and the course of the infection pattern could be observed. Thereby, it was also possible to group uncharacterized CpGV-M genes according to their transcriptional profile. In contrast, a delayed and limited transcriptional activity of CpGV-M genes was observed in midguts of CM strains resistant to CpGV-M. This indicated that CpGV-M is able to enter the midgut in resistant CM and start the viral transcription. This truncated infection does not result in a permissive infection of the host. In addition, the transcription of the resistant CM strain infected with the resistance overcoming isolate CpGV-I12 was followed by qPCR to proof if a successful infection of a resistant CM strain leads to the same course of infection as seen as in susceptible CMs. Six representative genes (*ie-1 lef-8, mcp, pe38, f-protein* and *granulin*) were chosen for this analysis. All of them showed the same course of infection in the resistant CM strain as seen in the susceptible CM strain.

Contributed paper. Tuesday, 9:30. **77**

Transmission of mixtures of insect pathogenic viruses in a single virion: towards the development of custom designed virus insecticides

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Alphabaculoviruses (lepidopteran nucleopolyhedroviruses) have a characteristic physical structure that facilitates the transmission of genetic diversity. We demonstrate that coinfection of *Spodoptera exigua* larvae by SeMNPV and a deletion genotype of SfMNPV resulted in the production of mixed virus occlusion bodies (OBs) containing both the parental viruses. This also occurred when phylogenetically more distant viruses were used: SfMNPV and AcMNPV coinfections in *S. frugiperda* larvae also resulted in mixed virus species OBs. Approximately half the virions present in OBs produced following coinfection with mixtures of different alphabaculoviruses contained both viruses, indicating that the viruses coinfected and replicated in a single cell, and were co-enveloped within the same virion. Serial passage experiments revealed that both viruses persisted in the mixed-virus population by coinfection of insects during several rounds of insect-to-insect transmission. These results have dramatic implications in alphabaculovirus evolution and ecology. This mixed virus production technology is the subject of a PCT (patent) and opens the way to the development of custom-designed insecticides for control of different species of caterpillar pests on crops.

Contributed paper. Tuesday, 9:45. **78**

Improvement of UV-resistance of Baculovirus by displaying the Nano-material binding peptides on the Polyhedron Envelope

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Baculoviruses are sensitive to UV radiation and this characteristic causes the control efficacy of viral insecticides unsteady in the fileds. The polyhedron envelope of baculoviruses, which is composed of carbohydrate and phosphorylated protein (PEP), is the first barrier against the disadvantageous environment. We found that orthologs of *Autographa colifornica multiple nucleopolyhedrovirus* (AcMNPV) PEP*,* such as *Helicoverpa armigera nucleopoly-hedrovirus* PEP, *Cydia pomonella granulovirus* Cp20 or Cp22 might not repair the absence of polyhedron envelope in the *pep*-knocked-out AcMNPV construct. The C-terminal (168~252aa) of AcMNPV PEP might deliever GFP to be expressed on the surface of polyhedron. Consequently, we had constructed the AcMNPV recombinants in which the C-terminal of PEP was fused with the peptides which might specifically bind melanin or nano-scale ZnO. These results may lay a foundation for developing intensive UV-resistant viral insecticides.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

BACTERIA 2

Contributed paper. Tuesday, 8:00. **79**

***Yersinia entomophaga* MH96 (Enterobacteriaceae) BC subcomplex of the Yen-Tc ABC toxin is able to induce toxicity independent of the A subcomplex**

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A novel gram-negative, rod-shaped, non-spore-forming bacterium, *Yersinia entomophaga* MH96 (Enterobacteriaceae), was isolated from diseased larvae of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). *Y. entomophaga* produces a proteinaceous toxin complex (Yen-Tc) that is responsible for mortality in a range of insect species, mainly within the Coleoptera and Lepidoptera. The Yen-Tc is made up of two chitinase subunits (Chi1 and 2) and five Yen subunits (A1, A2, B, C1, and C2). The TcA, B, and C subunits are related to members of the Toxin complex (Tc) toxin family, with orthologs identified from several other bacterial species including *Serratia entomophila* and *Photorhabdus luminescens*. Characterization of Yen-Tc pathology has revealed a progressive deterioration of the midgut epithelium of susceptible insects. Although the specific mechanism of Yen-Tc remains unknown, cellular and molecular work has begun shedding light on how the Tc family functions. The current model proposes that the TcA component binds to the cell surface and forms a pH-triggered channel that allows translocation of the TcBC subcomplex into the cytoplasm. Once in the cytoplasm the carboxy-terminus of the TcC subunit dissociates and becomes active, which causes toxicity in both insect and mammalian cells. A major component of this model is the requirement of an intact toxin complex in allowing TcBC to be transported into the cell. Based on our investigations of Yen-Tc, the YenBC subcomplex and the YenC subunit do not necessarily require full complex assembly to trigger cell toxicity. We will present and discuss our findings in relation to the current model.

Contributed paper. Tuesday, 8:15. **80**

Interaction of *Bacillus thuringiensis* Cry1Ab toxin with Mucus-rich structures

Diego Segond[1,2], Agnès Rejasse[1], Christophe Buisson[1], Shuyuan Guo[1,3], Karine Adel-Patient[2,4] , Hervé Bernard[2,4], Didier Lereclus[1], Christina Nielsen-LeRoux[1],
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Bacillus thuringiensis larvicidal Cry toxins are currently known for their strong host specificity; which is mainly due the presence of specific toxin binding sites on midguts of susceptible insect larvae. Meanwhile Cry toxins can also bind to compounds in the peritrophic matrix (PM) of several insects (*Rees *et al.* 2009; Valaitis and Podgwaite 2013). In *G. mellonella* infected with toxin alone, we observed structural modification of the peritrophic matrix but no evidence for the biochemical explanation for this modification is found so far. Knowing that "mucus" is along with chitin the main components of PM and that mucus is commonly found in several organisms, we aim to investigate the capacity of Cry1Ab to bind to several mucus rich structures. Indeed, our hypothesis is that the heavily glycosylated proteins (peritrophins and mucins) and proteoglycans shared by both vertebrate and invertebrate mucus may bind Cry toxins, therefore questioning on the "specificity" of these toxins used in GMO crops. Using, commercial pork stomach mucins, mice intestinal mucus, vertebrate cell-culture mucus and PM and peritrophins from *G. mellonella,* we then deeply analyzed Cry1Ab-mucus interactions. The presentation will deal with results from far western blot studies, ELISA binding experiments, inhibition ELISA with sugars, lectins or anti-Cry1Ab monoclonal antibodies. Identification of the interacting structure by LC/Ms/Ms analysis and resulting toxicity using insect and cellular models will be also shown.

*J Invertebr Pathol. 2009 Mar; 100(3):139-46; J Invertebr Pathol. 2013 Jan; 112(1):1-8.

Contributed paper. Tuesday, 8:30. **81-STU**

Pore formation helping ability and binding affinity of BmABCC2 and BtR175 against Cry1A toxins.

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By *in vitro* toxicity assay using Sf9/Baculovirus expression system, we previously provided a novel evidence that *Bombyx*

mori ABC transporter C2 (BmABCC2) functions as a receptor for Cry1A toxins. We also demonstrated that BmABCC2 can confer approximately 10-1000 times higher suscetibility to the cells than cadherin-like receptor (BtR175) and BmABCC2 and BtR175 co-expression exerts synergistic effect in susceptibility confering ability. This synergistic effect sugested that these two receptors have different roles in the mode of action of Cry1A toxins in Sf9. Thus, we addressed to find the difference in the roles of the two receptors. First, we evaluated pore formation helping ability of the receptors using xenopus oocyte expression system and the two-microelectrode voltage clamp technique. When Cry1Aa or Cry1Ab toxin was administrated to BmABCC2 expressing oocytes, current continuasly increased during toxin incubation, indicating that pores were continuasly assembled on the cell mambrane. However, when BtR175 expressing oocytes were administrated with toxins current increment speed was lower than in BmABCC2 expressing oocytes, indicating that BtR175 has lower function than BmABCC2 in pore formation helping. In contrast, BmABCC2 and BtR175 co-expressing oocytes showed at least 4 times higher current increment speed than BmABCC2 single expressing oocytes. This indicates that synersism occurs at least in part in the pore formation process, although synergistic effect is very low in comparison to that seen in Sf9 expression sysytem. We also compared the binding affinity to Cry1A toxins of BmABCC2 and BtR175 using Biacore systems. We will discuss these results, too.

Contributed paper. Tuesday, 9:00. **82**

A necessary step in the mode of action of the Cry8 toxin: the elimination of DNA from the Cry toxin-DNA complex

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Several crystal (Cry) proteins are known to occur as DNA-protein complexes. However, the role of the DNA associated with the activated toxin in the mechanism of action of the Cry toxin has long been ignored. Here, we focused on the DNA-activated Cry toxin complex. Both forms of the Cry8Ca2 and Cry8Ea1 toxins, i.e., with or without bound DNA, were separately obtained. Size-exclusion chromatography analysis indicated that the Cry8Ca2 toxin-DNA complex has a tight or compact structure. The Cry8 toxin-DNA complex is more likely to move toward the air/water interface and is more hydrophobic than the toxin without DNA. Competitive binding assays indicated that the Cry8Ca2 and Cry8Ea1 toxins without DNA specifically bind to the midgut of *Anomala corpulenta* and *Holotrichia parallela* larvae, respectively. In contrast, the association of DNA with each toxin might result in the nonspecific recognition of the Cry toxin and its target receptor in the insect midgut. The association of the DNA fragment with the Cry8 toxin was shown to protect the Cry protein from digestion by proteases. Based on our results, we propose an additional step in the mechanism of action of the Cry8 toxin and elucidate the function of the associated DNA as well as the importance of the removal of this DNA for the insecticidal activity of the toxin.

Contributed paper. Tuesday, 9:15. **83 STU**

How does the *Bt* Cry41Aa toxin kill human cancer cells?

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In this study the cytocidal activity associated with the Cry41Aa human cancer cell-active toxin of *Bacillus thuringiensis* (*Bt*), also known as Parasporin-3, was characterized. We investigated the effects of recombinant Cry41Aa on the human hepatic cancer cell line HepG2 to elucidate its mode of action. Cry41Aa shares structural homology with commercially used insecticidal toxins. The fact that some *Bt* toxins are able to kill mammalian cells may threaten the use of *Bt* toxin-based pesticides in the future. Moreover the preferential and narrow cytocidal activity of Cry41Aa has potential for anticancer drug design. Significant uptake of fluorescent dye was observed in susceptible cells as little as 10 minutes post administration, suggesting rapid membrane damage. Microscopic observation revealed cellular and nuclear swelling induced within the first hour of treatment. The activation of apoptosis effectors Caspase 3/7 was not observed within 24 hours, although phosphorylation of p38 MAP kinase was. Our results suggest that Cry41Aa, like its insecticidal homologues - but unlike some other Parasporins, is a pore-forming toxin that rapidly increases membrane permeability in the target cell. Research is on-going to identify whether a specific receptor is present on the surface of susceptible cells.

Contributed paper. Tuesday, 9:30. **84 STU**

Which regions of the *Bt* Cry41Aa toxin are responsible for its activity against human cancer cells?

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The *Bacillus thuringiensis* human cancer cell-active Cry41Aa toxin (Parasporin3) contains the five conserved sequence blocks found in many insecticidal toxins and is believed to possess the same three domain fold. However, Cry41Aa is predicted to have an extra loop in its domain II as well as an additional “ricin” domain at its C-terminus. Deletion of the “ricin” domain resulted in a stable protein with a toxicity to HepG2 cells not significantly different to the non-modified toxin. Several deletions of the loop region all resulted in an unstable protein that could not be further analyzed. Various bioinformatic procedures were used to identify the loops at the apex of domain II that have previously been implicated in receptor binding in the insecticidal Cry toxins. A range of mutations in the putative loop 1 were made but none affected toxicity to HepG2. In loop 3 the presence of an aromatic residue at position 509 was found to be important for toxicity. In an attempt to further dissect which regions are important for toxicity hybrids have been made between insecticidal and cancer cell-active toxins. Our data to date suggest that Cry41Aa has a mechanism of action similar to the three-domain insecticidal Cry toxins.

Contributed paper. Tuesday, 9:45. **85**

Parasporin PS1Aa2 induces ionic channels in lipid bilayer membranes and calcium oscillations in sensitive cells

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Parasporins are *Bacillus thuringiensis* Cry toxins that are active against tumor cells. Like many Cry toxins, parasporin

PS1Aa2 (Cry31Aa2) formed pores in artificial membranes. These pores had several levels of conductance; the most frequently observed in 150 mM KCl solutions were of 11, 16 and 21 pS. Microspectrofluorometric experiments with the Fura-2 probe showed that the presence of PS1Aa2 can induce changes in intracellular calcium levels, most often in the form of calcium oscillations and sometimes as sustained increases. Such responses were observed in the presence and absence of extracellular calcium, with the tumor cell lines HeLa and HepG2, and with the non-tumorous cell line HEK 293. Calcium oscillations have not been described previously for Cry toxins even though some studies have shown that calcium appears to be involved in their mode of action. Our experiments required the use of much higher concentrations of toxin than suggested from the published cytotoxicity results. Despite the presence of fragments previously identified as active, its low efficacy appears to be related to the presence of DNA in the preparations causing the protein to precipitate. Future work aimed at elucidating the origin of these calcium oscillations and their role in toxicity will be greatly facilitated by an improvement in the method of preparation of this toxin.

Contributed paper, 10:00. **86-STU**

***Caenorhabditis elegans – Bacillus thuringiensis* interactions: new insights into mechanisms of host resistance and pathogen virulence**

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Genetically tractable model nematode *Caenorhabditis elegans* has been successfully used in the host-pathogen interaction studies and helped to uncover conserved virulence factors of clinically relevant pathogens. At the same time interactions of this nematode with its natural pathogens are poorly investigated. Bacteria from the genus *Bacillus* are among potential natural pathogens of the nematodes. Therefore, previously we isolated 768 *Bacillus* strains and tested them for the virulence to nematodes. Although only 3% of tested *Bacillus* strains were pathogenic, one strain called *B. thuringiensis* DB27 exhibited extreme virulence to *C. elegans*. Currently we are trying to tackle both sides of this host-pathogen equation and aiming to identify virulence factors of *B. thuringiensis* DB27 as well as *C. elegans* defense mechanisms. First, combining plasmid-curing and genome sequencing, we discovered that novel nematicidal Cry21 toxins with synergistic activity are the main nematicidal factors of DB27. We expressed these novel toxins in *E. coli* and confirmed their activity against *C. elegans*. Importantly, these toxins are also active against other free-living and animal parasitic nematodes, suggesting their potential application against parasitic nematodes. Our parallel work on the host side led to the discovery of *C. elegans* novel innate immunity pathway involved in the defense against pathogens. Specifically, we identified novel function for Dicer in *C. elegans* antibacterial innate immunity and showed that this function is largely associated with microRNA processing. Taken together, our reciprocal studies uncovered a previously unknown role for DCR-1/Dicer in *C. elegans* antibacterial immunity as well as identified novel nematicidal toxins.

SYMPOSIUM 4 (Viruses) Tuesday, 10:30-12:30

Small non-coding RNAs as regulators of insect host-virus

Symposium. Tuesday, 10:30. **87**

Role of cellular and virus-encoded microRNAs in insect host-virus interactions

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MicroRNAs (miRNAs) are small non-coding RNAs of ~22 nucleotides which play significant roles in gene regulation at transcriptional as well as post-transcriptional levels. They are produced in almost all eukaryotes and also encoded by some viruses. Besides cellular miRNAs that may participate in anti-viral responses following infection, virus-encoded miRNAs may target host genes to interfere with host survival, proliferation and immunity. Furthermore, virus-encoded miRNAs may regulate replication of virus to avoid over replication and quick demise of the host or facilitate virus entry into persistent infection. The interaction may become more complicated in the presence of third parties, such as microbiota and endosymbionts, that in turn may affect the host's miRNA profile and indirectly disturb virus replication. In the presentation, the role of miRNAs in mosquito-arbovirus interactions with a reference to the effect of Wolbachia as an endosymbiont on the interactions will be discussed.

Symposium. Tuesday, 11:00. **88**

Sensing viral RNA in *Drosophila melanogaster*

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RNA interference plays a central role in antiviral innate immunity in flies. Indeed, flies mutant for the three key components of the small interfering (si)RNA pathway, namely Dicer-2, R2D2 and Argonaute (AGO) 2 are highly sensitive to a wide range of viruses (1). Dicer-2 produces virus derived-siRNAs from viral RNAs throughout its RNaseIII activity. The Dicer-2/R2D2 heterodimer then loads these siRNAs onto AGO2 in the RNA-induced silencing complex, RISC. The RISC complex is then able to target viral RNAs, thus impairing the ability of the virus to successfully replicate. Although *in vitro* and *in vivo* experiments clearly indicate that Dicer-2 can process long double stranded RNA, the exact nature of the viral RNAs sensed *in vivo* in infected cells remains mysterious. We are interested in understanding how Dicer-2 senses viral RNAs, with a particular focus on the contribution of the N-terminal DExD/C helicase domain, which is conserved in mammalian RIG-I like receptors. Indeed, *in vitro* experiments have revealed a critical role of this domain in both processivity of the enzyme and discrimination of the extremities of the template RNA (1,2). To address this question, we take advantage of a combination of approaches including *Drosophila* genetics, next-generation sequencing technologies and bioinformatics analysis.

(1) Kemp *et al.*, J. Immunol. 2013
(2) Cenik *et al.*, Mol Cell. 2011

Symposium. Tuesday, 11:30. **89**

Small RNA-directed antiviral immunity in disease-vector mosquitoes

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The natural maintenance cycles of many mosquito-borne pathogens require establishment of persistent non-lethal infections in the invertebrate host. While the mechanisms by which this occurs are not well understood, antiviral responses directed by small RNAs are important in modulating the pathogenesis of viral infections in disease vector mosquitoes. Infection of Aedinine vector species with viral pathogens triggers the production of short interfering (siRNAs) and another class of virus-derived small RNAs, ping-pong-dependent piwi- interacting RNAs (piRNAs). Unlike ping-pong-dependent piRNAs that have been described previously, from repetitive elements or piRNA clusters, our work suggests biogenesis in the mosquito soma. Similar to siRNAs, viral piRNAs also appear capable of modulating the pathogenesis of viral infections in mosquito cells. Thus, the non-canonical piRNA pathway present in the soma of Aedinine vector species may provide robustness to the primary siRNA-based antiviral response.

Symposium. Tuesday, 12:00. **90**

Controlling viral infection in in insects

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The paradigm held for a long time that RNAi response in most metazoans does not undergoes an amplification step and only acted cell-autonomously has prevailed in the model system Drosophila melanogaster and it is widely accepted for higher metazoans. The absence of systemic RNAi spread in Drosophila was directly tested in one study that used dsRNA-expressing transgenes in vivo in flies. We challenged this idea by identifying a dsRNA uptake pathway in Drosophila and showing that flies defective in several of the RNAi uptake genes are hypersensitive to virus infection, indicating that RNAi uptake is essential in the process of antiviral defense. In a second area, using a cloning approach to capture small RNAs with a 5' triphosphate group, we show that virus-derived siRNAs (vsRNA) bearing 5' triphosphate group accumulate in Sindbis virus (SINV) infected Drosophila melanogaster, suggesting that secondary vsRNA are produce during infection. Finally, we found that Criket Paralysis virus encoding RNAi suppressor, CrPV-1A specifically interacts with Ago-2 within assembled holo-RISC, without modifying RISC composition, and that this interaction prevents RISC cleavage of target mRNAs. Interestingly, we discovered that CrPV1A recruit an E3 ligase. The implication.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

MICROBIAL CONTROL 1

Contributed paper. Tuesday, 10:30. **91**

Double trouble for thrips: Effective biopesticide combinations to control soil-dwelling stages in chrysanthemums

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Western flower thrips (WFT) are pests of global significance and a constant challenge in greenhouse floriculture. Faced with a lack of conventional control products, Canadian growers have embraced the use of biological control strategies to manage this pest. Soil-dwelling stages of thrips (pro-pupae, pupae) can be targeted with different natural enemies, including biopesticides. *Steinernema feltiae* (e.g., Nemasys®), applied as a drench, is widely used. *Metarhizium brunneum* (formerly *anisopliae*; Met52™) has recently been registered in Canada and the granular biopesicide product is incorporated into potting media. A series of trials were set up to assess compatibility of the two control agents, and the relative efficacy of individual and combined, i.e. nematode plus Met52, treatments against WFT. Fewer *S. feltiae* were recovered from Met52 treated soils over time than from untreated media; this was generally accompanied by a concurrent increase in the number of free-living nematodes recovered. The rice carrier in the biopesticide may have served as a food source for the free-living nematodes, promoting population growth, which may have affected survival of *S. feltiae*. The individual nematode and fungus treatments had a measurable suppressive effect on thrips, but the combined nematode/fungus treatment provided superior control throughout. WFT populations were consistently lower on plants receiving the combined treatment and significantly fewer WFT (< 2 per plant) were found on the plants at the conclusion of the trial (8 weeks). Opportunities therefore exist to enhance the reliability and cost-effectiveness of thrips biocontrol strategies by taking an integrated approach to the deployment of biopesticides.

Contributed paper. Tuesday, 10:45. **92-STU**

Lethal and sub-lethal impacts of fungal biopesticides on house fly populations in simulated field settings of biocosms

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Management strategies for control of house flies in poultry houses include cultural, biological and chemical tactics; however, use of broad-spectrum chemical larvicides and adulticides is the only reliable tool for poultry growers to manage 'fly burst' situations. Our aim was to exploit post-eclosion resting behaviors of teneral flies to evaluate the population control potential (lethal and sub-lethal impacts) of oil formulations of *Beauveria bassiana* and *Metarhizium*

anisopliae under simulated field settings called 'biocosms'. Experimental biocosms were created in plastic boxes where the vertical walls were fitted with sprayed plastic sheeting (blank oil or conidia in oil). A cohort of 300 fly pupae was added to each biocosm; on emergence, the adult flies moved to the vertical surfaces to harden their wings, simulating the exposure likely to occur in the fields. The biocosms were monitored daily for mortality and enumeration of egg laying and egg viability until all adult flies had died. Fungal treated biocosms resulted in 100% mortality within 10-17 days, depending on the fungal species. Treated populations also showed significant reduction in egg viability and life-time fecundity. Furthermore, >20% reduction in basic reproductive rate (B0) was observed in treated fly populations. Together these results demonstrate that application of oil formulations of entomopathogenic fungi could suppress existing fly populations and substantially reduce population growth rates in poultry houses as part of an IPM program. Further studies will focus on evaluating fungal persistence on typical structural surfaces, optimizing application parameters and validating these strategies under actual field conditions in poultry houses.

Contributed paper. Tuesday, 11:00. **93-STU**

Management of *Prostephanus truncatus* (Horn.) on stored maize using *Beauveria bassiana* (Bals.)

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Control of the larger grain borer (*Prostephanus truncatus* Horn) using chemical insecticides is no longer desirable due to environmental and food safety issues. Classical biological control using the Histerid beetle, *Teretrius nigrescens* has been adapted in several locations in Ghana. However, *P. truncatus* is still causing tremendous losses of stored maize. There is a growing interest in using mycopesticides to complement other integrated pest management measures. Recent research in the UK has identified *Beauveria bassiana*, IMI 389521 as a suitable control agent for grain storage pests in the UK. In this study, the pathogenicity of *B. bassiana* IMI 389521 was evaluated against adults of *P. truncatus, Sitophilus zeamais* and *T. nigrescens*. The result obtained from the study indicates that *B. bassiana*, is pathogenic against adults of *P. truncatus* and *S. zeamais. Teretrius nigrescens* was less susceptible to the fungus. To determine the most effective concentration of *B. bassiana* for the control of *P. truncatus* in a semi-field trial, a laboratory dose response experiment using four concentrations of *B. bassiana*, ($1x10^8$ to 10^{11}/kg maize) was studied. Successful control of *P. truncatus* on infested maize was achieved at $1x10^{10}$conidia per kg maize. Semi-field trial to evaluate the efficacy of *B. bassiana*, against *P. truncatus* on maize stored on cobs (dehusked) and on shelled kernels is on-going. The availability and safety of maize will be enhanced, through reduction in the use of chemical insecticides if the isolate is proved effective thereby improving the livelihood of smallholder farmers in Ghana.

Contributed paper. Tuesday, 11:15. **94-STU**

Lack of involvement of chitinase in direct toxicity of *Beauveria bassiana* exudates to the aphid *Myzus persicae*

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Insect disease causing *Beauveria bassiana* produces a range of insecticidal metabolites and enzymes such as chitinases and proteases although few have been shown to be toxic simply through contact. Using supernatant from broth cultures of a single strain, *B. bassiana* could cause significant mortality of green peach aphid, *Myzus persicae,* within 24 hours of application. Three day old broth cultures were the most effective, with less insect mortality seen using 6 day old broth. However, aphicidal activity increased again for 7 day old broth. Submerged cultures grew better and produced stronger aphicidal supernatants when cultured in media with start pH above 5.5. Chitinase was produced a day earlier than protease Pr1. The enzymes, however, appeared to have little impact on aphicidal mortality given that their peak production periods do not correlate with the aphicidal activities of 3 or 6 day old cultures. Supernatants treated with EDTA and heat respectively, still killed aphids. High quantities of hydrolytic enzymes produced using insect cuticle medium showed no aphicidal activity. No beauvericin nor bassianolide, two known insecticidal metabolites, were detected in the supernatants. The identities of the key aphicidal components of the *B. bassiana* supernatants thus remain to be resolved.
Keywords: supernatant, *Beauveria bassiana*, chitinase, Pr1, aphicidal, EDTA, beauvericin, bassianolide

Contributed paper. Tuesday, 11:30. **95-STU**

Entomopathogenic fungi for control of false codling moth in South African citrus orchards

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False codling moth, *Thaumatotibia leucotreta* Meyrick (1912) (Lepidoptera: Tortricidae) is a key economic pest of citrus in South Africa causing pre- and post-harvest damage. Soil microbes, such as entomopathogenic fungi, offer an additional means of controlling this pest by targeting the soil-dwelling life stages. Three fungal isolates, two of the species *Metarhizium anisopliae s.l.* and one of the species *Beauveria bassiana s.l.* caused the highest levels of mortality of *T. leucotreta* fifth instar larvae in laboratory bioassays. In addition, these isolates were capable of persisting in a citrus orchard for six months, in sterile soil, whilst still remaining infective towards *T. leucotreta* fifth instar larvae. Since results may differ substantially under field conditions, further research was undertaken to determine whether these isolates remained effective when applied to non-sterile soil beneath the canopy of citrus trees in an orchard. A field trial consisting of one hectare treatment blocks, and a smaller caged trial were initiated to address this issue. Fungal spores were applied via spraying in an aqueous suspension at a concentration of $1x10^{14}$ spores per hectare for the field trial and at three different concentrations for the caged trial. Results of the large scale field trial, four months post application, support the

persistence capability of these isolates and, suggest that, although all three isolates were capable of reducing *T. leucotreta* infestation in comparison to the control block, *B. bassiana* performed best with an 81.33% reduction. It is thus suggested that future trials focus on the performance of this isolate.

Contributed paper. Tuesday, 11:45. **97-STU**

Wireworm control with entomopathogenic fungi and plant extracts

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Wireworms, the soil-dwelling larvae of click beetles, cause severe damage in arable crops and vegetable production. Currently, no registered and effective insecticides are available. The development of alternative control approaches including botanicals or insect pathogenic organisms are demanded and favoured by EU legislation (Directive 2009/128/EC). Limited efficacy of already tested entomopathogenic fungi (EPF) could be improved by synergistically acting botanicals. In the present study repellency of botanicals towards the wireworm species *Agriotes lineatus* and potential interactions of the most effective repellent with a wireworm-infecting fungus strain (*Metarhizium brunneum)* was investigated. Behaviour and mortality of wireworms were assessed in two-dimensional terraria (40cm x 50cm x 0.6cm) with a peat-sand substrate in a choice test for up to three weeks. Wireworm location was recorded and locomotion trails were manually traced, photographed and trail length determined on the treated and untreated half of the terrarium. We found that the garlic extract R3 repelled wireworms at rates of 1.2 g/L substrate, while this concentration hardly reduced efficacy of the EPF strain. Thyme oil was comparably repellent, but also strongly antifungal. The EPF strain was not repellent. Potential synergies between EPF and efficacy enhancing botanicals will be discussed for a biological control strategy.

Contributed paper. Tuesday, 12:00. **98-STU**

Long-term persistence of *Beauveria brongniartii* BIPESCO 2 used for cockchafer control in the Euroregion Tyrol

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The fungus *Beauveria brongniartii* (Sacc.) Petch has been used to control the European cockchafer *Melolontha melolontha* L. for more than two decades. The goal of this study was to assess persistence of the applied *B. brongniartii* strain in the soil of 20 cockchafer infested sites in East, North and South Tyrol. The sites have been treated with Melocont® Pilzgerste (BIPESCO 2) at different frequencies and time points during the last 20 years. At all sites the denistiy of *M. melolontha* larvae decreased from high levels at the start of treatments to levels below the damage threshold at the time point of sampling in 2012. A selective medium was used to determine *B. brongniartii* density and recover *B. brongniartii* isolates from soil samples. Collected isolates were subjected to genetic analyses to discriminate the applied strain from naturally occuring strains. Highest densities of *Beauveria* spp. (up to 6.8 x 10^5 CFU g^{-1} soil dry weight) were detected in soils which have been treated with Melocont® Pilzgerste at least three times during the last three years (3 sites) prior to the sampling date. BIPESCO 2 was detected in 7 sites of which one was treated for the last time15 years prior to sampling. *Beauveria* spp. density varied strongly among and within fields and in 71% of the 162 soil samples no *Beauveria* was detected. Results suggest that periodic applications of the *B. brongniartii* biological control agent increase density and persistence of the fungus in soil and support a long-term control of *M. melolontha*.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

DIS. OF BENEFICIAL INVERTEBRATES 1

Contributed paper. Tuesday, 10:30. **99**

The Curious Case of the PaV1 in Adult Caribbean Spiny Lobsters

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The pathogen PaV1 (Panulirus argus Virus 1) exacts a heavy toll from juvenile Caribbean spiny lobsters with an estimated 24% in Florida dying of it before they reach maturity and recruit to the fishery. Prevalence is also high among adult populations, especially in the northern Caribbean (e.g., Puerto Rico – 17%). However, PaV1 manifests differently in adults. They may act as "carriers" because adults rarely develop visible infections and do not seem adversely affected by the pathogen. Infected adults are not avoided by healthy conspecifics, as occurs among juveniles. Moreover, adult females with subclinical PaV1 infections are often captured from the wild with a spermatophore or fertilized eggs, indicating that males are willing to mate with them. Adults with subclinical infections of PaV1 are not infectious to other adults or to the more susceptible juveniles. Although postlarval lobsters infected with PaV1 occur in the nearshore waters of Florida, experiments indicate that vertical transmission of PaV1 from females to embryos is not the mode of transmission. Instead, postlarvae acquire PaV1 shortly after arriving inshore from the oceanic plankton. These recent results suggest that PaV1 may be of little consequence to adult lobsters in contrast to its major effect on juvenile ecology and population dynamics. Just how adult lobsters retain subclinical infections of PaV1 remains a mystery.

Contributed paper. Tuesday, 10:45. **100**

Defining lobster-pathogen interactions via high-throughput gene expression studies: The discovery and description of the interplay between the American Lobster (*Homarus americanus*) and the ciliated parasite *Anophryoides haemophila*

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The American lobster (*Homarus americanus*) fishery is the economic engine for hundreds of coastal communities in Atlantic Canada and represents the last remaining significant wild fishery in Canada. Lobsters appear remarkably resistant to microbes in their natural environment however they are susceptible to the opportunistic ciliated pathogen *Anophryoides haemophila*, the causative agent of bumper car disease, during live holding. We have completed numerous controlled experimental infection studies to define the gross, histopathology, biochemical and molecular responses of lobster to this ciliated parasite. Recently completed high throughput oligonucleotide microarray and RNA-Seq transcriptomics studies have revealed a more comprehensive understanding of the molecular pathogenesis of disease in this unique lobster – parasite interaction. One caveat is interpreting the overwhelming wealth of bioinformatic data generated. This issue will be explored in the context of current annotation limitations for both arthropods and protistan parasites.

Contributed paper. Tuesday, 11:00. **101-STU**

Metabolomic investigation of Bitter Crab Disease in snow crabs (*Chionoecetes opilio*)

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Bitter crab disease (BCD) is a fatal disease of crustaceans caused by parasitic dinoflagellates of the genus *Hematodinium.* This emerging disease has been reported in over forty species of crustaceans world-wide including several commercially important crustacean species. In Atlantic Canada BCD occurs in snow crabs (*Chionoecetes opilio*) off the northern coasts of Newfoundland and Nova Scotia. In the late stages of this disease, the dinoflagellate parasites proliferate within the hemolymph and hemal spaces within the crustacean's organs, with no apparent cellular inflammatory response to the infection. The cause of death in cases of BCD is presumed to be metabolic and osmotic dysregulation. In this study, we use a combination of untargeted and targeted metabolomic approaches to characterize some of the metabolic changes associated with BCD.

Contributed paper. Tuesday, 11:15. **102-STU**

Assessment of immunocompetence in the shore crab, *Carcinus maenas*, to natural exposure of pathogens

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UK populations of the shore crab *Carcinus maenas* host various pathogen assemblages. In particular, two geographically close but distinct populations in Weymouth, (Newton's Cove and Harbour), demonstrated entirely different pathogen profiles. Immune biomarkers were used to assess the immunocompetence of individuals in these populations in relation to their pathogen burden. Selected immune genes included *carcinin*, (antimicrobial peptide), *peroxinectin* (cell adhesion molecule and osponin) and the zymogen *prophenoloxidase,* (cleaved to form active *phenoloxidase,* involved in the melanisation of many invading pathogens). Immune gene expression was quantified using real-time PCR. Histopathology revealed greater pathogen incidence in Newton's Cove (95%) compared with Harbour (37%) and a high dissimilarity in the pathogen profile (82.61% SIMPER) between sites. Host immune expression in relation to the presence and absence of pathogens and number of different infections per crab, revealed significant (p=<0.01) differences in transcription between populations, suggesting site-specific factors also influenced immune expression. In addition, host RNA quality was compared between pathogen groups ('viruses', 'bacteria', 'macroparasites' and 'no pathogens' groups). Further analysis may reveal whether RNA degradation is a function of pathogen type within the host. This is the first study to compare immunocompetence and histopathology between different *Carcinus maenas* populations in the wild.

Contributed paper. Tuesday, 11:30. **103-STU**

Effects of artificial infection of juvenile edible crabs, *Cancer pagurus* with the parasitic dinoflagellate, *Hematodinium* sp.

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Parasitic dinoflagellates of the genus, *Hematodinium*, are thought to be significant pathogens of a wide range of crustaceans. Much is known of the ecology and effects of this disease on the sustainability of crustacean populations but significantly less is known about the mode of transmission and fate of infected animals. Attempts have been made to transmit the disease under aquarium conditions to several species of crabs resulting in a great deal of variation in mortality levels and the timescale of disease progression. To determine if *Hematodinium* infections are significant drivers of mortality in juvenile edible crabs (*Cancer pagurus*), crabs were injected with either 1 x 10^5 *Hematodinium* trophonts from an infected animal or sterile saline. Crabs were bled every four weeks to determine the progression of infection and its effects on the numbers of circulating haemocytes. Thirty three percent of the *Hematodinium*-injected crabs became infected and mortality occurred between 93 and 378 days post-challenge. Infected crabs appeared to moult less frequently than their uninfected counterparts but mortality did not appear to be directly caused by *Hematodinium,* as there was no significant difference in the mean time to death between infected and uninfected crabs. Both *Hematodiunium*-infected and uninfected crabs exhibited infections by a number of other disease causing agents including haplosporidium-like parasites, fungi and bacteria. These appeared to be key drivers of the mortality observed. These studies, albeit carried out on small cohorts of edible crabs, imply that *Hematodinium* is not a driver of host mortality at least under aquarium conditions.

Contributed paper. Tuesday, 11:45. **104**

A role of polychaetes in transmission of white spot syndrome virus in shrimp ponds?

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White spot disease (WSD) is caused by white spot syndrome virus (WSSV) (*Nimaviridae*). WSSV emerged in the early-to-mid 1990s in Southeast Asia and became panzoonotic since. The disease can be mitigated by introducing rigorous sanitation protocols, proper pond management, use of specific pathogen-free shrimp and by early diagnosis followed by eradication. The virus is transmitted horizontally by healthy individuals predating on diseased ones, via feeding on detritus or by intake of WSSV-contaminated water. WSSV can also be transmitted vertically via broodstock. The virus infects a wide range of crustaceans beyond the penaeids such as crabs and crayfish, and these co-inhabitants of ponds form a reservoir of WSSV for disease transmission to penaeids. Much less knowledge is there on the potential of resident benthic organisms as vectors for WSSV. A literature survey indicates that WSSV is present in a number of non-crustacean invertebrates, which sometimes vector the disease to penaeid shrimp. *Dendronereis* spp. is a most ubiquitous resident annelid in shrimp ponds and used as food source for shrimp. We showed that WSSV replicates in *Dendronereis* spp. and can be transmitted from this polychaete to penaeid shrimp. Furthermore there appears to be a positive correlation between the past incidence of WSD in ponds and the occurrence of WSSV in resident *Dendronereis* spp, whereas there is no correlation with other pond parameters. We hypothesize that *Dendronereis* spp., as a replicative host for WSSV, may serve as a reservoir for WSSV and may be associated with the persistence of this virus in pond systems.

Contributed paper. Tuesday, 12:00. **105**

Novel Pattern Recognition Receptor Protects Shrimp from *Vibrio* Infection by Binding Flagellin and LPS through Different Recognition Modules

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Pattern recognition receptors (PRRs) recognize pathogens thorough the pattern recognition modules. For example, Toll like receptors recognize the ligands through leucine-rich repeats (LRRs), and C-type lectins bind to glycans on the surface of pathogens by the C-type carbohydrate recognition domain (CRD, also called C-type lectin like domain CTLD). Many PRRs contain more than one kind of modules. In the present study, we identified a novel PRR, named *Leulectin*, which contains several LRRs and a CTLD. Such unique arrangement has not been found in any other organisms. Recombinant Leulectin and the modules (LRRs and CTLD) were found to protect shrimp from *Vibrio* infection. An ELISA-based screen was performed to identify the potential ligands the two modules may recognize. Results showed that LRRs could recognize the *Vibrio* flagellins, and CTLD could recognize lipopolysacchraides (LPS). The Leulectin-flagellin interaction was determined by the third LRR of Leulectin and the N-terminus of flagellin, and the Leulectin-LPS interaction was dependent on the long loop region of CTLD in a calcium-independent manner. The ligand-recognizing activity of LRRs and CTLD was critical for Leulectin to bind to bacteria, and the binding was the basis for Leulectin to protect shrimp from bacterial infection. This study clearly showed the interesting synergy between distinct modules of a PRR.

Contributed paper. Tuesday, 12:15. **106**

Observations on *Agmasoma penaei* and *Perezia nelsoni* in White shrimp *Litopenaeus setiferus* from the Gulf of Mexico

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In June 2012 a few shrimp from Plaquemines parish LA with the symptoms of microsporidiosis were delivered to the Louisiana Aquatic Diagnostic Laboratory for identification. Light microscopy including examination of Luna-stained paraffin sections, and electron microscopy showed the infection was limited to ovaries and was caused by a microsporidium producing roundish pansporoblasts with 8 spores (3.6 x 2.1µm) and anisofilar (2+6) polar filaments, the features corresponding to the diagnosis of *Agmasoma penaei* (=*Thelohania penaei* Sprague 1950, n.comb Hazrad and Oldacre, 1973). Comparison of the SSUrDNA sequence of the novel isolate to *A.penaei* from Thailand revealed 95% similarity, which suggests these geographical isolates, may be two different species, a conclusion supported by several ultrastructural dissimilarities and different tissue tropism. Phylogenetic analyses places this species as a divergent taxa within the clade IV (microsporidia of terrestrial origin) sensu Vosbrinck, Debruner-Vossbrinck, 2005. In two shrimps infection of ovaries with *A. penaei* was accompanied by heavy infestation of muscles with another microsporidium *Perezia nelsoni. P.nelsoni* produces individual spores (2.0 x 1.1µm). Structurally and genetically (SSUrDNA sequence similarity >99%) LA isolate was identical to *Perezia nelsoni* from the Mississipi coast of the Gulf (Canning et al., 2002). Previously reported infection of muscles with *A.penaei* may be due to overlooked double infection with *P.nelsoni.* Supported by Louisiana Department of Wildlife and Fisheries.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

FUNGI 3

Contributed paper. Tuesday, 10:30. **107**

Comparison of ecological traits of co-existing *Metarhizium*: What does it take to dominate an agricultural field?

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It is expected that particular adaptive ecological traits influence species abundance and distribution within an ecosystem. We evaluated selected traits of different co-existing species and genotypes of the entomopathogenic fungi *Metarhizium* isolated from an agroecosystem in Denmark. Fifteen fungal isolates representing 11 genotypes were tested for: UVB tolerance, *in vitro* growth at 12.5°C and 21.5°C, mycelial growth from the insect cadaver into the surrounding soil, virulence and conidia production on cadavers. The results showed that the relative performance of the most abundant *Metarhizium* genotype was intermediate for mycelial growth in soil and in vitro growth at 12.5°C / 21.5°C while it showed high UVB tolerance and conidia production compared to other genotypes. We discuss whether the two latter traits are most important to dominate the *Metarhizium* community in agricultural habitat or whether the "Jack of all trades" performance could be the key to understand the dominance of a particular genotype.

Contributed paper. Tuesday, 10:45. **108-STU**

Effect of entomopathogenic fungal strains on non-target arthropods in sour cherry orchard

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Efficacy of *Metarhizium* and *Beauveria* entomopathogenic fungal strains for the control of cockchafer grubs was evaluated in sour cherry orchards. Safety like possible effect of the inoculum on natural soil microbiota as well as efficacy and fate of these fungi need to be investigated. The applied fungal strains have wide host range, thus we have to determine the risks of their use during repeated long-term applications. Different inoculation methods were compared and the persistence of inoculum was monitored in the soil and on target and non-target organisms. The treatments were applied 2 times (May and July) in the space rows and we used pitfall traps as sampling method. Samples were collected 8 times during the summer of 2013. The samples were processed in laboratory and the numbers of different arthropods (collembolans, mites, thrips, flies, ants, spiders, centipedes, crickets, rove beetles, ground beetles) were recorded in each sample. The comparison of un-treated and treated areas, and the microscopical examination showed no significant differences in the frequency of species. As a conclusion, the effect of these entomopathogens on non-target arthropods is minimal and as such they do not impose any environmental risk.

Contributed paper. Tuesday, 11:00. **109-STU**

Potential of endophytic *Beauveria bassiana* in grapevine against insects

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Fungal entomopathogens are important antagonists of arthropod pests and have attracted increased attention as biocontrol agents in integrated pest management programs. In addition, evidence has accumulated that some entomopathogenic fungi like *Beauveria bassiana* (Bals.) Vuill. can endophytically colonize plants and provide a systemic protection against insect pests. Currently, it is unknown whether *B. bassiana* can exist as an endophyte in grapevine, *Vitis vinifera* (L.) and still maintains its antagonistic potential against insect pests. In the present study, the antagonistic activity of *B. bassiana* (strain ATCC 74040) after plant inoculation and endophytic establishment in grapevine against the vine mealybug *Planococcus ficus* was assessed using surface sterilized leaves for a bioassay. Possible effects of endophytic *B. bassiana* on the feeding preference of black vine weevil *Otiorhynchus sulcatus* choosing between control and inoculated plants was examined through choice assays. A significant effect of endophytic *B. bassiana* on growth during the whole observation period and on mortality of mealybugs one week after initial settlement was evident. Adult *O. sulcatus* chose significantly more often the control plants as a host plant compared to grapevine plants with endophytic *B. bassiana.* In addition, a microarray analysis was performed to get insights into genetic mechanisms behind the plant-fungus-interaction. The results indicate an up-regulation of diverse defense related genes in grapevine due to the endophytic establishment of *B. bassiana*. In conclusion, endophytic establishment of the entomopathogenic fungus *B. bassiana* in grapevine might represent an alternative and sustainable plant protection strategy, with the potential for reducing pesticide applications in viticulture.

Contributed paper. Tuesday, 11:30. **111**

Horizontal transmission of entomopathogenic fungi by ectoparasitoid *Habrobracon hebetor*

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Horizontal transmission of entomopathogens by parasitoids is well known for viruses but did not registered for fungi. Our experiments were carried out on the laboratory system *Galleria mellonella* (Lepidoptera, Piraliidae), – *Habrobracon hebetor* (Hymenopera, Braconidae) – *Beauveria bassiana* (Hypocreales, Cordycipitaceae). We found out that contamination of *H. hebetor* ovipositor with low titers of conidia *B. bassiana* and following envenomation of *G. mellonella* larvae led to mycoses followed by host colonization and conidiaformation. In addition *H. hebetor* females transmitted fungal conidia from infected (6 hours post inoculation with conidia) to native *G. mellonella* larvae, and this transmission led to successful mycosis of native host larvae. The decreasing of cellular and humoral immune reactions, significant increasing of adhesion and germination of fungus on cuticle of envenomated larvae were registered. As a result susceptibility of envenomated *G. mellonella* larvae to fungal infection was increased in thousands times compared with native control. Thus the paralyzation and strong inhibition of immune reactions of larvae by venom of *H. hebetor* allows to minimize quantity of transmitting with parasitoid fungal inoculum. We assumed that «paralyzing» parasitoids can take part in transmission of entomopathogenic fungi particularly in out-of-the-way places (shelters) as well as disperse of fungal infection under low density of hosts.

Contributed paper. Tuesday, 11:45. **112**

Fast spread of the parasitic *Laboulbenia formicarum* in a supercolony of the invasive garden ant *Lasius neglectus*

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Many ant species are highly successful invaders and can dominate vast areas by forming dense networks of connected nests in contrast to the smaller and discrete, spatially dispersed colonies of most social insects. However, it was recently proposed that such supercolonies are more vulnerable to infection by parasites and diseases as they would serve as large targets with high rates of transmission from nest to nest. Here we studied the invasive garden ant *Lasius neglectus*, a new pest species which is currently spreading throughout Europe where several populations are infected with the ectoparasitic fungus *Laboulbenia formicarum*. In one population (supercolony) we followed the prevalence and intensity of the infection over 10 years, revealing an epizootic spread of the ectoparasite with the mean annual prevalence increasing from 0.126 to 0.997. Distinct body parts of the ants had markedly different infection intensities, and at low intensities antennae and thorax were free from signs of infection. There were no seasonal differences in infection intensity and no other *Lasius* species in the area was found to be infected. These results give the first direct support to the hypothesis that supercolonies of invasive ants potentially face a significantly higher threat from parasites and diseases compared to ants with normal colonies, implying interesting perspectives for biological control of these pest species.

Contributed paper. Tuesday, 12:00. **113**

The dietary preference of a beneficial predator in apple orchards reveals an undocumented spore dispersal mechanism for entomopathogenic fungi.

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In the course of a floristic and ecological study of the entomopathogenic fungi found in apple orchards and strawberry fields (part of the IMBICONT biological control project), we observed resting spores in the frass of the earwig *Forficula auricularia*, a beneficial predator in apple orchards. The presence of resting spores in earwig frass suggests that in addition to being a beneficial predator, earwigs may play a role in the dispersal of Entomophthoromycota—a spore dispersal mechanism not previously documented for this group of fungi. In the lab, we observed that earwigs avidly consumed entomophthoromycotan-infected insects even while the fungus was actively ejecting conidia. We hypothesize that this fungus-insect meal might confer a nutritional benefit but that earwigs avoid foraging on insects infected by generalist entomo-pathogenic fungi (e.g. *Metarhizium*, *Beauveria*) because these generalist entomopathogens pose a risk that would potentially outweigh any nutritional benefit. We present the preliminary results from a series of choice-experiments to test these hypotheses.

Contributed paper. Tuesday, 12:15. **114**

Effects of entomopathogenic fungi on the "*Trialeurodes vaporariorum – Encarsia formosa*" system: preliminary results

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The effects of a selected group of six entomopathogenic fungal isolates (including the mycoinsecticide Naturalis and the *Beauveria bassiana* ATCC74040 strain contained into the commercial product) on the system "*T.vaporariorum* - *E.formosa*" were evaluated, considering the direct effect on the parasitoid development but also on the *E. formosa* activity and behaviour. The effect of fungal treatments on the parasitoid development was evaluated submitting infested tomato plants to the fungal treatments at different times from the parasitization and recording the parasitization rate and the parasitoids emergence. Then, the effect of fungal isolates on *E. formosa* behaviour and activity was examined in "free-multichoice" and "no-choice condition". Finally, the role of *E. formosa* in trasmitting the mycoses from infected to uninfected host population was estimated. Results showed that fungal treatments can affect the *E. formosa* development, particularly when applied before the parasitoids introduction and using the mycoinsecticide Naturalis. *E. formosa* showed no differential tropism in "free - multichoice" conditions and it was not able to locate and select the uninfected hosts "at distance" but it was able to detect and avoid infected hosts by direct exploration. Furtheremore, *E. formosa* was able in vectoring the fungal propagules from contaminated to uncontaminated hosts trough its activity. Results of these laboratory experiments provided important information about the possibility to integrate the entomopathogenic fungal treatments and the *Encarsia formosa* releases and clarified some biological and behavioural aspects of the "host–pathogen–parasitoid" system.

WEDNESDAY - 6 August

SYMPOSIUM 5 (Microbial Control) Wednesday, 8:00–10:00

Developments/Issues in the Regulation of Microbial Products: Harmonization across Jurisdictions

Symposium. Wednesday, 8:00 **115**

The authorisation and regulation of microbial biopesticides: why bother?

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The use of microbial biopesticides and other minimal-risk products is starting to become more widespread as a result of new government legislation that aims to reduce the excessive use of conventional chemical pesticides and increase the use of "alternative" control methods. In the European Union, a paradigm shift in pesticide policy has occurred recently with the enactment of the Sustainable Use Directive on pesticides. This legislation makes IPM mandatory for farmers and growers and gives specific emphasis to biologically based controls including microbial biopesticides. There has been significant recent activity in the biopesticides industrial sector, with multinational agchem / agri-business companies buying up biopesticide

companies. The large financial resources of the global companies should lead to an increase in the effectiveness, sales and availability of microbial biopesticide products, but SMEs will still have a critical role to play through the development of innovative, "next generation" biopesticides. All of these products will need to have authorization for use by government regulatory organisation. Authorization can be slow and expensive, which can be a barrier to product development. The authorities recognize this and have put in place measures designed to improve the system, sometimes with mixed results. We have explored why product authorization is necessary, and in this presentation we will discuss ways in which biopesticide regulation could be improved further.

Symposium. Wednesday, 8:24 **116**

Registration of Biopesticides in the EU: a company perspective

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One characteristic of many biopesticides is their narrow host range. This represents a lot of advantages, e.g. protecting the beneficial fauna, harmlessness towards human health etc., but it significantly reduces the potential market size for such products. The procedures for the registration of a biopesticide are mostly the same as for a chemical plant protection product, even if the characteristics of the active substance and the exigencies for the risk assessment differ in many aspects. The difficulties with registering biopesticides are often unknown or inappropriate data requirements, lack of experience within authorities to assess biopesticides, often resulting in unreasonable delays of the evaluation procedures, and too high registration fees. Under such conditions it is almost impossible for the industry to make the development of biopesticides with small-sized markets cost-effective. Furthermore they jeopardize investments in research for new biopesticides. Although the new EU regulation 1107/2009 provides new criteria for the approval of plant protection products - stricter evaluation timelines, a low risk category and evaluation within distinct zones- the uncertainties and high costs for the registration of biopesticides still exist. As a consequence, the industry will focus its investments in research and development of new biopesticides outside of the EU, where the registration of biopesticides is easier. It will become more difficult for European growers to have access to new, innovative and environmental friendly biopesticides in the future, especially in niche markets.

Symposium. Wednesday, 8:48 **117**

Biopesticide registration, a company perspective and how registration influences biopesticide R&D approach of companies in North American

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When developing a new bioinsecticide active and associated formulations there are a series of stage gates that must be met by R&D to ensure final product registration in North America. At each gate confidence is gained to justify further resourcing. This presentation will discuss in general terms the critical milestones and strategy for prioritizing those milestones for a bioinsecticide. Specific reference will be made to Met52 and R&D's role in generating knowledge for its current registration and label expansion. By presenting this perspective, it may shed light on the long path from discovering a promising isolate to making it a tool for growers. This may also help improve the dialogue between industry and academia to identify points along this path where collaborations can contribute towards that common goal.

Symposium. Wednesday, 9:12 **118**

Registration of biopesticides: how research can be structured to suit microbial registration needs and promote the commercial development of new biopesticides

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Invertebrate pathology is an applied field, and a major aim of research is to make the technologies discovered available to growers through the development and registration of new biopesticide products. The biopesticide market is growing at over 15% per annum: the global market was valued at $1.3 billion in 2011 and is predicted to reach $3.2 billion by 2017. There is a challenge therefore to meet the forecast demand for biopesticide products. Most of the micro-organism based products currently on the market are the ‚back catalogue', representing research and technology that has been on the laboratory bench for the last 20 or 30 years. To bring plant protection products to the market they have to be registered, how this happens varies country to country and can take many years. In a biopesticide commercialisation pathway, registration is a significant barrier, demanding considerable investment in time and financial resources. Biopesticide research projects need to be designed and structured so research and industry can work in alignment and so reduce the hurdle of registration. This presentation will explore approaches that have been implemented in biopesticide projects to better align research and industry objectives and build partnerships to facilitate the regulatory process thus reducing commercialisation costs and reducing product development timelines.

Symposium. Wednesday, 9:36 **119**

Current developments and issues on regulation of biopesticides- Lessons from REBECA project, comparison of EU and USA systems

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Microorganisms as active ingredients in plant protection products are gaining more and more importance. This is due to the fact that most of them have little effect (if any at all) on human health, non-target organisms, and the environment. However, registration of Microbial Plant Protection Products is still facing particular problems, which is partly due to the fact that some data requirements which can be easily covered for synthetic chemicals cannot be fulfilled for microorganisms and their products for technical reasons. On the other hand, the major advantage of most microbial products is that the microorganism species are scientifically well known and humans are familiar with them either through direct use or environmental exposure for a long time. Data requirements are similar in different regulatory systems, but acceptance of publicly available data for the risk assessment by authorities varies over time and between different regulatory systems.

BACTERIA 3

Contributed paper. Wednesday, 8:00 **120**

Resistance alleles to *Lysinibacillus sphaericus* are co-select in a *Culex quinquefasciatus* colony and display distinct features

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Two alleles of the *cqm1* gene, containing mutations associated to resistance to the Binary (Bin) from *Lysinibacillus sphaericus,* were co-selected in a laboratory resistant colony of *Culex quinquefasciatus* (R2362). The goal of this study was to identify these alleles and to analyze the homozygous larvae for each one, through different approaches. The alleles named $cqm1_{REC\ and}$ $cqm1_{REC\text{-}2}$ are characterized by distinct mutations, however, they code for transcripts of truncated proteins that are not located in the midgut epithelium and cannot act as receptors for the Bin toxin. Homozygous larvae for each allele show high resistance to the Bin toxin, low specific binding of Bin toxin to midgut microvilli proteins and low transcription level of the both resistance alleles. Their frequency in the R2362 colony showed that the $cqm1_{REC}$ has predominated during a long period (> 100 generations), however, it has been replaced by the $cqm1_{REC\text{-}2}$ that became the most frequent allele. A colony established from the cross of homozygous individuals from each allele (1:1 ratio) showed that $cqm1_{REC}$ assumed a higher frequency, compared to $cqm1_{REC\text{-}2}$, during a period of 21 generations. An AS-PCR-screening detected the presence of $cqm1_{REC\text{-}2}$ allele in larvae from field populations and its frequency and distribution was lower than that found for $cqm1_{REC,}$ suggesting that this allele has a higher risk to be selected. The fitness cost of individuals homozygous is under study to evaluate the impact on the biological performance of individuals carrying these alleles.

Contributed paper. Wednesday, 8:15 **121-STU**

Untangling insect pathogenicity in plant-beneficial pseudomonads by a combination of comparative genomics, bioassays and histopathology

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The Pseudomonas fluorescens group harbors many root-associated plant-beneficial bacteria that suppress soil-borne fungal diseases and promote plant growth. Remarkably, two strains, Pseudomonas protegens CHA0 and Pseudomonas chlororaphis PCL1391, additionally display oral insecticidal activity towards lepidopteran larvae. This ability is associated with the Fit insect toxin and unknown GacA-regulated traits. However, the exact course of infection, the target organs and the virulence factors beyond Fit are yet undiscovered. To tackle these open questions we combined various methods. Fifteen strains of fluorescent pseudomonads, including four new isolates, were characterized for both their plant-beneficial traits and their insecticidal activity. Whereas the former were found throughout the entire *P. fluorescens* group, the latter was restricted to strains of *P. protegens* and *P. chlororaphis*. By next generation sequencing and subsequent comparative genomics we identified a small set of genes common to all insecticidal strains, but absent in non-insecticidal strains. These genes could therefore encode potential virulence factors against insects. Histopathology to detect affected insect tissues and fluorescence microscopy to localize the bacteria during the infection complete this study which reveals intriguing aspects on insect pathogenesis of plant-associated pseudomonads and identifies several strains with potent dual activity against root pathogens and insect pests.

Contributed paper. Wednesday, 8:30 **122**

Comparative analysis of the Cqm1 and Aam1 ortholog proteins from mosquitoes that have a differential capacity to bind to the Binary toxin from *Lysinibacillus sphaericus*

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The Cqm1 and Aam1 are ortholog proteins from the midgut of *Culex quinquefasciatus* and *Aedes aegypti* larvae, respectively. These related proteins, with 74% of identity, are expressed as membrane-bound alpha-glucosidases and, functionally, Cqm1 also acts as the receptor of the insecticidal Binary (Bin) toxin from *Lysinibacillus sphaericus*, while Aam1 does not. The major goal of this study was to analyze some features of these proteins produced in Sf9 cells. The recombinant proteins obtained in this expression system showed the same molecular weight and kept their differential capacity to bind to the Bin toxin, as the native proteins. The Cqm1 sequence presents three predicted N-glycosylation sites (PGS), however, the analysis of the recombinant protein suggested that it does not have glycans. On the other hand, Aam1 sequence has six PGS and analysis of the recombinant protein showed that four of them contain carbohydrates that can be removed by the glycosidase PNGase F. Site-directed mutagenesis of these PGS prevented the insertion of carbohydrates and these mutant proteins did not bind to the Bin toxin, similarly to the wild Aam1. In terms of their catalytic function, both recombinant proteins displayed alpha-glucosidase activity and Aam1 showed a two-fold increase compared to Cqm1. Analysis of protein sequences showed that one segment of the Cqm1, that is required for Bin toxin binding, is not conserved in the Aam1 and might be an important factor for their differential capacity to interact with the Bin toxin and, thus, for the refractoriness of *Ae. aegypti* larvae to *L. sphaericus*.

Contributed paper. Wednesday, 8:45 **123**

Resilience of the intestinal epithelium to the action of a bacterial pore-forming toxin and to xenobiotics in *Drosophila*

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The host defense against pathogens encompasses two complementary arms: i) resistance, attacking directly the pathogen, which is mediated by the immune system; ii) resilience, also referred to as tolerance, withstanding and repairing damages inflicted either by the pathogen or by the host's own immune system. We have discovered that the compensatory proliferation of *Drosophila* intestinal stem cells (ISCs) allows the intestinal epithelium to maintain its homeostasis during *Serratia marcescens* infection, and thus constitutes a *bona fide* resilience mechanism. Resilience is not limited to the control of ISC proliferation. Within three hours of ingestion of *S. marcescens*, the epithelium becomes very thin in the absence of cell death. Strikingly, epithelial cells are able to recover their shape and volume in the next 6-9 hours. Attack by *S. marcescens* hemolysin, a 2 nm-wide pore-forming toxin, leads to the controlled extrusion of the cytoplasm of epithelial cells. This may help in purging the cytoplasm from damaged organelles. We have initiated a molecular analysis using both a genetic and a transcriptomics approach and thus identified tens of genes required for the regeneration phase. One of them, a conserved cyclin of previously unknown function, plays a major role noncell-autonomously and is required for the expression of early response genes. Many of these genes are also induced by exposure to xenobiotics such as caffeine. We have found that the cyclin mutants are more susceptible to the ingestion of caffeine. Thus, we may have uncovered a novel stress response pathway that underlies a new resilience mechanism.

Contributed paper. Wednesday, 9:00 **124**

Cadherin mutations and Bt resistance: Field screening and fitness costs

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Insecticidal crystal toxins from *Bacillus thuringiensis* (Bt) of the Cry1A family bind to a 12-cadherin domain protein in the midgut of lepidoptera and eventually form pores in the midgut epithelium, leading to death of the insect. Mutations in this cadherin confer Cry1A resistance to several Lepidoptera. In the course of an F1 screen to estimate the frequency of such mutations in field populations of the tobacco budworm *Heliothis virescens*, a novel mutation was found. Like the first mutation found in this species, it is caused by insertion of a transposable element, but in a different location. Allele frequency changes were recorded over several generations of artificial selection for a homozygous mutant strain, showing a substantial fitness cost to knockout cadherin mutations, even under optimal conditions in the laboratory. Although this type of transposon-induced mutation may be moderately common in field populations, its high fitness cost makes it unlikely to threaten the sustainability of transgenic cottin expressing Cry1A toxins.

Contributed paper. Wednesday, 9:15 **125**

Down regulation and mutation of cadherin gene associated with Cry1Ac resistance in Asian corn borer

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Development of resistance in target insects is a major threat to long-term use of transgenic Bt crops. To delay the evolution of resistance in target insect through the implementation of the effective strategies, it is fundamental to understand the pests' resistance mechanisms. One of the most important mechanisms of insect resistance to Bt crops is the alteration of interaction between Bt toxin and its receptor in the insect midguts. Asian corn borer (ACB), *Ostrinia furnacalis*, is a key pest of maize to be targeted by Bt maize. A Cry1Ac resistant strain of ACB has been established in the laboratory. Compared to the membrane proximal extracellular region (MPR) of cDNA of *ofcad* that encodes a cadherin-like protein in ACB from the susceptible strain, there were three mutant alleles of *ofcad* (MPR-r1, MPR-r2, and MPR-r3) associated with resistance to Cry1Ac toxin. Each of those mutant alleles had 2-3 aa substitution in the putative-toxin binding region (TBR) of the cadherin, especially $Thr^{111} \rightarrow Ser^{111}$ was accurate. In addition, MPR-r2 had a deletion expected to eliminate 26 aa-residues in TBR, which resulted in decline in the binding of MPR to Cry1Ac in the resistant strain compared to the susceptible strain. Furthermore, down regulation of ofcad was associated with Cry1Ac resistance, response to the stress of low level Cry1Ac toxin in susceptible strain. These results suggest that Cry1Ac resistance in ACB is primarily associated with the down regulation of *ofcad*. Mutations in *ofcad* resulting in amino-acid substitutions and deletions might mediate higher level of resistance.

Contributed paper. Wednesday, 9:30 **126**

ABCC transporters mediate insect resistance to multiple Bt toxins revealed by BSA analysis

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Insect resistance to *Bacillus thuringiensis* (Bt) is one of the main threats for the long term use of Bt-based products, including Bt crops. Identification of genes conferring resistance to Bt will contribute to delaying the development of resistance as well as to provide additional information about the mode of action of these bacteria and its insecticidal toxins. By using linkage analysis based on high throughput sequencing, we have found a novel type of mutation in the ABCC2 transporter conferring resistance to Bt. In addition we have also found that different members of the ABCC transporters can act as receptors for not only Cry1A toxins but also for the Cry1C type toxins. The identified mutation in the ABCC2 transporter is

localized in a region that does not physically interact with the toxins but in the intracellular ATP-binding domain instead. Our toxin binding studies have revealed that such mutation correlates with a reduction in toxin insertion into the membrane (irreversible binding) and suggests that ABCC activity as transporter is necessary for the proper action of Bt toxins.

CONTRIBUTED PAPERS Wednesday, 8:15-9:45

DIS. OF BENEFICIAL INVERTEBRATES 2

Contributed paper. Wednesday, 8:15 **128**

Nosema ceranae News: Update on Species Competition and Host-Pathogen Interaction Studies

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The apparent recent invasion of *Nosema ceranae* and its dominance over *Nosema apis* in honey bee populations in the USA and elsewhere have presented both an enigma and a treatment problem for apiculturists and scientists. Several studies, including those of our research group, have shown that *N. ceranae* produces more mature spores than *N. apis,* and we demonstrated that reproduction of *N. ceranae* recovers more quickly from fumagillin treatment than does *N. apis.* In addition, *N. ceranae* hyperproliferated in the presence of very low fumagillin concentrations in laboratory bioassays. Proteomic-level studies of fumagillin-*N. ceranae*-honey bee interactions continue and we are investigating the mechanisms of protein regulation in response to infection and fumagillin treatment. In studies of infectivity, we found that *N. ceranae* consistently has a higher IC_{50} than *N. apis.* The effect is most pronounced at 1 day post eclosion. We investigated the interaction of *N. ceranae* and *N. apis* in individual bees and found that *N. apis* produced more spores than *N. ceranae* in 62% of bees infected with equal dosages of both *Nosema* species. Mixed species infections negatively affected survival time (15-17 days) compared to single species infections (20 and 21 days for *N. ceranae* and *N. apis,* respectively) and uninfected bees (27 days). Midgut spore counts were higher for mixed species infections than for single species infections, but we did not find evidence that *N. ceranae* outcompetes *N. apis* in an individual host..

Contributed paper. Wednesday, 8:30. **129**

Influence of temperature on the development of _Nosema apis_ and _Nosema ceranae_

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Nosema apis and *Nosema ceranae* are two fungal pathogens infecting the European honey bee, *Apis mellifera*. These obligate intracellular pathogens, belonging to the phylum Microsporidia, infect epithelia cells of the midgut and elicit nosemosis. Recent studies suggested that *N. ceranae* is more virulent than *N. apis* and can lead to severe colony losses. These colony losses are so far only reported from the Southern parts of Europe. In the Northern parts (e.g., Denmark, Sweden, Finland, and Germany) *N. ceranae* could not be correlated to colony losses so far. While *N. ceranae* seems to have replaced *N. apis* in the bee population in South Europe, this is not the case for the Northern parts of Europe. Both findings suggest a climatic angle for spread, assertiveness, and virulence of *N. ceranae*. Exact whether parameters as temperature or humidity, which hinder or favor *N. ceranae* infections, are not determined so far. Spanish colleagues recently showed that *N. ceranae* has a better adaptation to complete its endogenous cycle at warmer temperatures. However, the results based on *in vivo*-infections only give a minor hint on different proliferation of both obligate intracellular pathogens exposed to different temperatures. We here present our results on the intracellular development of *N. apis* and *N. ceranae* exposed to different temperatures using our recently established cell culture model for *Nosema* spp.

Contributed paper. Wednesday, 8:45 **130-STU**

The involvement of bumblebee small interfering RNA pathway against two different bee viruses

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Wild pollinators such as bumblebees are in global decline. They share a pathogen network with other pollinators, consisting of multi-hosts pathogens and multi-pathogens hosts. Disturbance of these associations could lead toward the further host decline. Insects have developed certain immune pathways to combat viruses, of which the small interfering RNA pathway (siRNA) is important. By unveiling the interaction of the virus with the host defense pathway we can better understand the virulence of certain viruses in specific hosts. Here we use two viruses, israeli acute paralysis virus (IAPV) and slow bee paralysis virus (SBPV), representing two infection types after injection in *Bombus terrestris*, i.e. IAPV presents an overt acute infection resulting in mortality, while SBPV results in a covert persistent infection. First, to determine viral replication dynamics by following the negative and positive strands, we developed a new method in combining multiplex ligation-dependent probe amplification and qPCR. The results show both viruses experienced an exponential-plateau phase, and their replication strand were relatively low compared with genome (positive) stand. Second, both viruses increased the expression of Dicer-2 and SID, thereby activating siRNA. Finally we performed small RNAs sequencing to screen if differences in the siRNA production could explain different viral virulence.

Contributed paper. Wednesday, 9:00 **131**

Impact of Wolbachia endosymbionts on the evolution of sex determination in the isopod Armadillidium vulgare

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Terrestrial isopods are crustaceans that represent a major component of the litter ecosystem, as they mainly feed on dead plant material and participate in litter decomposition. In the isopod *Armadillidium vulgare*, genetic sex determination follows female heterogamety (ZZ males and ZW females). However, many *A. vulgare* populations harbor maternally-inherited *Wolbachia* bacterial endosymbionts. These bacteria are reproductive parasites that convert genetic males into phenotypic females, leading to populations with female-biased sex ratios. The W sex chromosome has been lost in lines infected by *Wolbachia* and all individuals are ZZ genetic males. The female sex is determined by the inheritance of *Wolbachia* by the *A. vulgare* individual. Surprisingly, some *A. vulgare* lines exhibit

female-biased sex ratios despite the lack of *Wolbachia*. In these lines, female individuals are ZZ genetic males carrying an unknown feminizing factor. To elucidate the genetic basis of female sex determination in these lines, we sequenced the genome of a female by Illumina technology. After *de novo* genome assembly, we identified a large piece of the *Wolbachia* genome transferred into the *A. vulgare* nuclear genome. The transferred genomic fragment co-segregates perfectly with the female sex in pedigrees. These results suggest that sex determination in these *A. vulgare* lines is under the control of nuclear gene(s) of bacterial origin and that bacterial reproductive parasites can drive shifts in sex determination mechanisms in animals. This research is funded by an ERC Starting Grant (EndoSexDet) to RC.

Contributed paper. Wednesday, 9:00 **132**

First characterization of a mollusk beta pore forming toxin

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Aerolysins are virulence factors belonging to the beta pore-forming toxin (b-PFT) superfamily that are abundantly distributed in bacteria. More rarely, b-PFTs have been described in eukaryotic organisms. Recently in our laboratory, a putative cytolytic protein called Biomphalysin have been characterized in the snail, *Biomphalaria glabrata*, who's primary structural features suggest that it could belong to this b-PFT superfamily. We have showed that, despite weak sequence similarities with aerolysins, Biomphalysin shares a common architecture with proteins belonging to this superfamily. A phylogenetic approach revealed that the gene encoding Biomphalysin could have resulted from horizontal transfer. Its expression seems to be restricted to immune-competent cells and is not induced by parasite challenge. Recombinant Biomphalysin showed hemolytic activity that was greatly enhanced by the plasma compartment of *B. glabrata*. We further demonstrated that Biomphalysin is able to bind to parasite and has a plasma dependent anti schistosomal activity. Surprisingly, investigation of *B. glabrata* genome reveals that this family appears to be multi-genic. More than 20 genes were identified suggesting an important role played by Biomphalysin proteins for *B. glabrata*.
These results provide the first functional description of a mollusk immune effector protein involved in killing of *S. mansoni*, agent of the second most widespread tropical parasitic disease after malaria.

Contributed paper. Wednesday, 9:30 **133-STU**

A first report of an immune-associated cytosolic PLA_2 in insects: Gene structure and function

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Eicosanoids are a group of C20 polyunsaturated fatty acids most derived from arachidonic acid (AA). A phospholipase A_2 (PLA_2) catalyses AA release from phospholipids at SN-2 position. Among three different groups of PLA2s ($cPLA_2$, $sPLA_2$, $iPLA_2$), only $sPLA_2$ (secretory type of PLA_2) has been identified as venom- or immune-associated functions. This study reports the first $cPLA_2$ (cellular and calcium-dependent PLA_2) in insects. A hemocyte transcriptome of *Spodoptera exigua* possessed 1 for $sPLA_2$, 2 for $iPLA_2$, 1 for $cPLA_2$. Expression of Se-$cPLA_2$ was inducible to bacterial challenge in hemocyte and fat body. RNA interference of Se-$cPLA_2$ expression significantly suppressed cellular immune responses of *S. exigua*. A recombinant of Se-$cPLA_2$ exhibited a specific enzyme activity influenced by p H, temperature, and calcium. Especially, Se-$cPLA_2$ was susceptible to a specific $cPLA_2$ inhibitor, but not to a specific $iPLA_2$ inhibitor. These results indicate that Se-$cPLA_2$ is a specific $cPLA_2$ and associated with immune responses.

CONTRIBUTED PAPERS Wednesday, 8:00-9:30

FUNGI 4

Contributed paper. Wednesday, 8:00 **134**

Fungal dimorphism in the entomopathogenic fungus *Nomuraea rileyi*: A search for *in vivo* produced quorum-sensing molecules

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Similar to other zoopathogenic fungi, many insect pathogenic hyphomycetes including species within the genera *Metarhizium*, *Beauveria*, *Isaria,* and *Nomuraea* exhibit a defined *in vivo* dimorphic developmental program. This program involves switching between apical to budding growth providing mycopathogens with both tissue-invasive and vegetative growth capabilities. The budding yeast-like vegetative cells absorb nutrients in the hemocoel without apparent damage to tissues allowing the insect to continue to feed and develop. The ability to switch cell phenotypes is crucial for successful *in vivo* development. *N. rileyi* exhibits a defined developmental program that involves the sequential production of cellular phenotypes designed to perform spatially and temporally unique functions. Upon reaching the nutrient-rich hemolymph the penetrant germ tube switches from an apical to a budding growth program leading to the formation of freely circulating hyphal bodies. The yeast-like hyphal bodies grow exponentially in the nutrient-rich haemolymph reaching densities that far outnumber circulating hemocytes. As a critical threshold density is achieved, these hemolymph-borne cells synchronously revert to an apical growth program forming the tissue-invasive cell phenotype. The ensuing mycelial phase produce and secrete a suite of metabolites that can modulate host development, that rapidly kill the host, and that efficiently digests insect tissue leading to the mummification of infected larvae. In this presentation investigation we detail the hyphal body to mycelial transition of *Nomuraea* in the insect host, provide evidence for quorum-sensing that is produced and released into the hemolymph, and detail the extraction and examination of the elicitors that mediate the dimorphic switch.

Contributed paper. Wednesday, 8:15 **135**

Multilocus genotyping of *Amylostereum* spp. associated with *Sirex noctilio* and other woodwasps from Europe reveal clonal lineage introduced to the US

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Sirex noctilio is a woodwasp of Eurasian origin that was inadvertently introduced to the southern hemisphere in the 1900s and to North America over a decade ago. It attacks various *Pinus* species and cause significant mortality in pine plantations. *Sirex noctilio* is associated with a symbiotic white rot fungus, *Amylostereum areolatum*, which females inject into trees when they oviposit and which is required for survival of developing larvae. We examined the genetic diversity of *A. areolatum* isolated from *S. noctilio* and other woodwasps collected from Europe in comparison with samples from northeastern North America to determine origin of introduction(s). Multilocus genotyping of nuclear ribosomal regions and protein genes revealed two widespread multilocus genotypes (MLGs) among the European samples, one of which is present in the US. The other US *S. noctilio*-associated *A. areolatum* represented unique MLGs, although variation was primarily due to the laccase gene, with the other loci having conserved sequences. The closest relative to these US strains is a German strain with identical ITS, mtssu and tef sequences. These findings indicate multiple introductions of *S. noctilio* to North America from Europe or from Europe via South America. Our results also showed lack of fidelity between wasp hosts and *Amylostereum* species, and we found a North American woodwasp carrying an *A. amylostereum* MLG likely introduced by *S. noctilio*. These results underscore the need to study North American siricids and their fungal symbionts as *S. noctilio* continues to spread in North America.

Contributed paper. Wednesday, 8:30. **136**

Preliminary analysis of the genome sequence of *Beauveria caledonica*

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Beauveria caledonica is a pathogen of a number of insects, especially Coleoptera. Occurrence has probably been under-reported due to the morphological similarity the ubiquitous entomopathogenic fungus *Beauveria bassiana.* Recent phylogenetic studies have shown that *B. bassiana sensu lato* is really a species complex. The genomic differences between species of *Beauveria* can assist understanding of the importance of selected gene in disease and ecology of these fungi. We report on initial comparisons of the genome of *B. caledonica* strain isolated in New Zealand and *B. bassiana.* The genome was sequenced using 3 lanes of a MiSeq by NZGL (New Zealand). 15,890,840 150-bp read pairs were obtained for the 32-Mb *Beauveria* strain (~149 fold coverage). After assembly using the programme ABySS, a total of 10,951 contigs were obtained over 39 bp and an N50 of 21676, with 2827 over 500 bp. Preliminary comparisons were conducted on a range of phylogenetic, secondary metabolite and mitochondrial gene regions. Assembly of the mitochondrial genome was used to assess completeness of the coverage. The genome sequence of *B. caledonica* shows significant divergence from *B. bassiana*.

Contributed paper. Wednesday, 8:45 **137**

MALDI-TOF Mass Spectrometry: A complement to sequence-based identification technologies for major fungal entomopathogens

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Matrix-Assisted Laser Desorption/Ionization Time of Flight mass spectrometry has been tested and proven to be a rapid and inexpensive approach closely replicating the results of gene sequence-based analyses to identify species in such major entomopathogenic fungal genera as *Metarhizium* and *Beauveria*. While MALDI-TOF cannot replace PCR-based approaches for identifications or phylogenetic studies and cannot demonstrate relationships among fungi, it does appear to be extremely valuable for rapidly detecting anomalous isolates that need further detailed PCR-based study. This mass-spectrometric technique may be extremely valuable for ecological and population biology studies, as well as offering significant support for the efficient curation of large culture collections holding hundreds to thousands of isolates for which verified MALDI-TOF profiles are available. In comparison to the results obtained from the more routine analyses of (still) small numbers of individual genes, MALDI-TOF uses large numbers of cell proteins to group samples and, therefore, monitors much larger proportions of a total organismal genome; evidence will be presented that such a more complete coverage of the total genome suggest the existence of biogeographical groupings that may not be easily detected by PCR-based studies.

Contributed paper. Wednesday, 9:00 **138**

Transcriptomic study reveals *Pandora formicae* expressing pathogenicity related genes in final stages of host infection

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Pandora formicae (*Entomophthorales*, Entomophthoro-mycota) is an obligate pathogen of the common red wood ant, *Formica rufa*. The fungus, similarly to other fungi of this group, enters the host body through cuticle, where it exploits nutritional resources within the haemocoel. When the infected ant is close to death, the fungus triggers a change in host behavior, manipulating it to climb a leaf (e.g. grass) or a twig. The fungus attaches the moribund host with rhizoids, the host legs grasp around the leaf or twig and the mandibles bite to vegetation and lock. Then the host dies and soon after the fungus breaks through the cuticle with conidiophores producing asexual spores. This quick transformation requires activity of several enzymes involved in cuticular breakdown, cell wall formation, and other processes. To study this, we have constructed transcriptome libraries of the last two stages: 1) when the ant is just dead with no fungal growth outside except the rhizoids, and 2) when external conidiophores are present. This first *de novo* transcriptome of an entomophthoralean fungus, in interaction with host, provides accurate insight into the plethora of genes expressed during final stages of infection, crucial for fungus transmission and reproductive success.

Contributed paper. Wednesday, 9:15 **139**

Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals putative virulence factors shared by fungal and oomycete entomopathogens

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The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae and therefore has been seen as a potential biological control agent against disease vector mosquitoes. However, little is known about the pathological process of *L. giganteum* in its mosquito host. In order to detail the molecular basis of entomopathogenicity, a transcriptome analysis was initiated for *L. giganteum*, using various Next Generation Sequencing technologies. Homology searches have led to the annotation of ca. 20,000 transcripts based on significant similarity to known proteins and revealed a full complement of plant pathogenic oomycete effector orthologs. The characterization of full-length transcripts corresponding to Cellulose Binding Elicitor Lectin (CBEL), Crinkler, and elicitin proteins demonstrated that *L. giganteum* is the first described animal pathogenic oomycete to secrete canonical Crinkler and CBEL effectors. In addition, phylogenetic analyses identified a Glycoside Hydrolase 5 (subfamily 27; GH5_27) as a putative virulence factor. Genome mining indicated that GH5_27 orthologs are shared by entomopathogenic oomycetes and fungi, but virtually absent in all other oomycetes and fungi. Using PCR, GH5_27 fragments were amplified and sequenced from additional entomopathogens, suggesting that oomycete and fungi underwent convergent evolution and that GH5_27 proteins may play a crucial role in insect/microbe pathosystems. Detailing the molecular basis of entomopathogenicity may allow for the use of oomycetes and fungi as control agents against insect pests, reducing the use of insecticides that can have negative impacts on the environment and human health.

SYMPOSIUM 6 (Bacteria) Wednesday, 10:30–12:30

Structure and Function of Novel Insecticidal Toxins

Symposium. Wednesday, 10:30 **140**

Structural and biophysical characterization of Cry34Ab1 and Cry35Ab1

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Bacillus thuringiensis strains are well-known for the production of insecticidal proteins upon sporulation and these proteins are deposited in parasporal crystalline inclusions. The majority of these insect-specific toxins exhibit three domains in the mature toxin sequence. However, other Cry toxins are structurally and evolutionarily unrelated to this three-domain family and little is known of their three dimensional structures, limiting our understanding of their mechanisms of action and our ability to engineer the proteins to enhance their function. Amongst the non-three domain Cry toxins, the Cry34Ab1 and Cry35Ab1 proteins are required to act together to produce toxicity to the western corn rootworm (WCR) *Diabrotica virgifera virgifera* Le Conte via a pore forming mechanism of action. Cry34Ab1 is a protein of ~14 kDa with features of the Aegerolysin family (Pfam06355) of proteins that have known membrane activity, while Cry35Ab1 is a ~ 44 kDa member of the Toxin_10 family (Pfam05431) that includes other insecticidal proteins such as the binary toxin BinA/BinB. The Cry34Ab1/Cry35Ab1 proteins are important solutions for control of WCR having been developed as insect resistance traits in commercialized corn hybrids for control of WCR. The structures of Cry34Ab1 and Cry35Ab1 have been elucidated to a resolution of 2.15 Å and 1.80 Å, respectively. The solution structures of the toxins were further studied by small angle X-ray scattering (SAXS) and native electrospray ion mobility mass spectrometry. We present here the first published structures from the Aegerolysin and Toxin_10 protein domain families.

Symposium. Wednesday, 10:50 **141**

Structure/function studies of Cry5B via alanine-scanning mutagenesis

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Cry5B is a three-domain crystal protein that attacks nematodes. In collaboration with the laboratory of Partho Ghosh, the three dimensional structure of Cry5B has been solved. Cry5B shows significant similarities with three-domain insecticidal crystal proteins in domains I and III but significant differences with insecticidal proteins in domain II. To better understand structure function relationships in Cry5B, we performed alanine-scanning mutagenesis of the entire toxin domain in which each point variant was tested in bioactivity assays with the free-living nematode *Caenorhabditis elegans*. Alanine point variants were classified into three classes—those with reduced/no bioactivity, those with relatively normal bioactivity, and those with increased bioactivity against *C. elegans*. More than 400 point variants were successfully tested. Some of those in the latter class (increased bioactivity) have been selected for further study, including fully quantitative analyses and testing their spectrum of increased action against other nematodes. Our results, as well as their implications for crystal protein – nematode interactions, will be presented..

Symposium. Wednesday, 11:10 **142**

Insights into the structures of non-3-domain toxins through structural modeling

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A knowledge of the structure of insecticidal toxins is a major benefit in elucidating mode of action and is, therefore, fundamental for targeted mutagenesis to test mechanistic hypotheses, to alter target range and increase toxicity. Crystal structures of activated 3-domain toxins, Cyt toxins, Vip2, Mtx1 and anthrolysin are available and can be used to model structures for related proteins. There remains a significant

number of potent invertebrate-active toxins that do not fall within these classes. Crystallography is labour-intensive, requiring large quantities of pure, mono-disperse protein and often proves difficult. Recent developments in the field of *in silico, ab initio* structural modelling allow the generation of models in the absence of related sequences in the protein structure database. This may allow us to predict protein structures and use these predictions to develop testable hypotheses for the modes of action of the toxins. This procedure has been applied to several non-3-domain toxins and toxin-associated proteins. For one such protein, a structure is proposed, consistent with a pore forming mechanism of action. Analysis of secondary structure content is consistent with this model and evidence of pore formation has been produced. Mutagenesis of a region known to be important in structurally-related toxins was shown to eliminate toxicity. While further study is clearly required, modelling, thus, allows us to predict and test hypotheses related to the mode of action of toxins for which experimental structures are, as yet, unavailable.

Symposium. Wednesday, 10:30 **143**

Novel MTX Toxins for Insect Control

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In addition to the conventional 3-domain Cry proteins, the Gram-positive bacteria Bacillus thuringiensis can also harbor other classes of insecticidal toxins with distinct structures, receptors, and modes of action. Among them are a group of proteins that share significant similarities to MTX2/3 toxins at the structural level, but are very divergent at the amino acid sequence level. In this presentation, we will discuss the general features of these MTX toxins, and agriculture applications for the control of insect pests.

Symposium. Wednesday, 11:50 **144**

Insecticidal toxins from *Photorhabdus luminescens* and *asymbiotica*, targeting the actin cytoskeleton and GTP-binding proteins

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Photorhabdus luminescens and *asymbiotica* live in the gut of entomopathogenic nematodes, which invade insect larvae, where they release the bacteria. Here, the bacteria produce toxins, which kill the insects. We studied tripartite Tc toxins from *P. luminescens* and a novel toxin (PaTox) from *P. asymbiotica*.
Tc toxins consist of three components TcA, TcB and TcC, which occur in several isoforms. TcA is responsible for the binding and up-take of the toxin, B is a linker and C carries the biological activity. Recent crystal structure analysis revealed a novel type of syringe-like injection mechanism, which depends mainly on TcA but needs all components (1). We studied the biological activity of TccC3 and TccC5, which are isoforms of TcC. TccC3 ADP-ribosylates actin at threonine148, thereby actin polymerization is enhanced (2). TccC5 ADP-ribosylates Rho proteins at glutamine61, a modification which persistently activates of Rho GTPases. Both modifications of actin and Rho proteins induce clustering of the actin cytoskeleton (2).
The *P. asymbiotica* toxin PaTox glycosylates Rho proteins by attaching GlcNAc at tyrosine32/34 (3). The modification inhibits Rho signaling, because Rho activation and interaction with effectors are blocked. In addition, PaTox harbors a deamidation domain, which activates heterotrimeric G proteins, including Gq/11 and Gi family proteins. Functional consequences of the actions of *Photorhabdus toxins* on actin and GTP-binding proteins are discussed.
References
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2. Lang et al. (2010) Science 327, 1139-1142.
3. Jank et al. (2013) Nat. Struct. Mol. Biol. 20, 1273-1280..

Symposium. Wednesday, 12:10 **145**

Molecular basis of parasporin-2 action toward cancer cells

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Parasporin-2 (PS2) is a crystal toxin isolated from parasporal inclusions of *Bacillus thuringiensis* A1547. PS2 has a strong cytocidal activity in liver and colon cancer cells without showing a typical insecticide. Accumulated molecular, cellular and *in vivo* experimental observations on PS2 indicate that the protein form a pore in membrane with a mega size assembly. The crystal structure of active PS2 monomer reveals that the protein elongates like a short rod, comprising almost β-strands. The polypeptide folding is similar to a class of aerolysin-like β-pore-forming toxins while there is no homology to insecticidal Cry toxins. N-terminal domain of PS2 is rich in aromatic residues and forms a groove which could be capable to grapple the target molecule. Amino acid substitutions of PS2 in the region indicate that the residues could be involved in cell-binding. The C-terminal domain contains β-sandwiches and the surface of the protein has a unique extensive track of exposed side chains of serine and threonine where thought be related to PS2 oligomerization and membrane pore formation. Single-particle EM analysis reveals that PS2 oligomer shows a ring shape with the 24nm length, 8 nm diameter and a 4nm pore while a structure of pore-forming aerolysin is the ring-like mushroom structure with a central pore. We would like to introduce current observations on anti-cancer toxin PS2 in vitro and vivo in this symposium.

CONTRIBUTED PAPERS Wednesday, 10:30-12:30

MICROBIAL CONTROL 2

Contributed paper. Wednesday, 10:30 **146**

Evaluation of the non-target effects of *Bacillus thuringiensis* subspecies *israelensis* in standardized aquatic microcosms

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Malaria, one of the most deadly vector-borne diseases in the world, is transmitted by the bite of an infected female *Anopheles* mosquito. *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) is a gram-positive, aerobic, spore-forming bacterium that produces crystalline inclusions that contain insecticidal proteins. *Bti* has been shown to be highly insecticidal to larvae of mosquitoes and blackflies, but is considered to have weak insecticidal activity against non-dipteran invertebrates in aquatic environments. Few studies have comprehensively studied the non-target effects of *Bti* under reproducible and standardized conditions. The objective of this study was to evaluate the effects of *Bti* on key non-target invertebrates in a highly reproducible synthetic multi-species system, the

standardized aquatic microcosm (SAM) system. The SAM system is initiated in a chemically defined medium with synthetic sediment and is inoculated with nine different species of photosynthetic microorganisms (PMOs) and different non-target invertebrates. Replicate SAMs were inoculated with a LD_{90} of *Bti* strain HD-522, whereas the control SAMs were not inoculated with *Bti*. Over a period of 2 months, the abundance of PMOs and invertebrates were determined by biweekly sampling. Differences between *Bti*-treated and control SAMs were assessed by statistical analyses of sampling data and biological diversity indices. The contributions of the SAM experiments to our understanding of the non-target effects of *Bti* are discussed.

Contributed paper. Wednesday, 10:45 **147**

Bacillus thuringiensis 00-50-5 strain with high activity against plant-parasitic nematodes and insect pests

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Plant-parasitic nematodes (PPNs) are serious pathogens of many other crops. Yellow cutworm Agrotis segetum is a serious insect pest of vegetable and grains. After liquid fermentation of Bacillus thuringiensis (Bt) strain 00-50-5, the cell-free supernatant (CFS) and the crystal protein toxins (CPT) have high activities against the root-knot nematode (RKN) Meloidogyne incognita and the yellow cutworm A. segetum. The mortality for second-stage RKN juveniles (J2) was 100% as early as 5 hrs after exposure to 0.909μg.mL^{-1} of dried CFS. The LC_{50} values were 0.037- and 0.015μg.mL^{-1} of partially purified Bt exotoxin at 5 hrs and 24 hrs after exposure, respectively. The mortality of A. segetum was 100% for first-instar larvae (L1) after exposure to 10μg.mL^{-1} CPT for 72 hrs. The LC_{50} value for A. segetum L1 was 0.417μg.mL^{-1}. An SDS-PAGE of the purified 00-50-5 CPT resulted in four main proteins with 133-, 60-, 27- and 25 kDa after treatment with 1%SDS, and three proteins with 133-, 60-, and 27-kDa after treatment with 0.1N NaOH. The Bt 00-50-5 has dual nematicidal and insecticidal activities against soil-dwelling pests, such as M. incognita and A. segetum.

Contributed paper. Wednesday, 11:00 **148**

Investigations on residues of *Bacillus thuringiensis* on tomato

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After an incidence of diarrhea in 2012, high concentrations of presumptive *Bacillus cereus* (including *B. thuringiensis* (*Bt*)) were found in German lettuce samples. Because of this incidence, in Germany a discussion about the risk of *Bt* residues started and is still ongoing.
To proof the degradation of *Bt* spores in glasshouses, experiments were conducted on tomato under laboratory, experimental field station and professional grower conditions. For all experiments the *Bt* product XenTari® was used.
In the glasshouse experiment with five applications of XenTari® applied in a weekly interval the concentration of *Bt* spores on tomato fruits ranged in all experiments between $4.9x10^4$ und $8.5x10^4$ cfu/ g fresh weight. For single application of *Bt* a max. spore concentration of $4.7x10^4$ cfu/g fresh weight was measured corresponding to the laboratory experiments and the experiments at a commercial farm. To proof the degradation *Bt* spores over time samples were taken after the last application over one week. Over all experiments the concentration of *Bt* spores was reduced up to only 46 to 77 % of the initial spore concentration within one week. A distinct reduction of *Bt* spores on fruits was achieved by modifying the application strategy. When only the upper parts of the tomato plant were treated with XenTari, a maximum concentration of *Bt* spores of 3.3×10^3 cfu / g fresh weight was recorded.

Contributed paper. Wednesday, 11:15 **149**

Biological control of western corn rootworm larvae (*Diabrotica virgifera virgifera*) with Dianem® (*Heterorhabditis bacteriophora*)

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The biocontrol product dianem® contains entomopathogenic nematodes, *Heterorhabditis bacteriophora.* It is officially registered in Austria as a plant protection product to control larvae of the Western Corn Rootworm (*Diabrotica virgifera virgifera*). Field results from Hungary, Austria and Italy applying 2×10^9 nematodes per ha obtained equally high control like chemical seed dressings with neonicotinoides or application of granular insecticides containing the pyrethroide Tefluthrin. Adapted application technology has been developed to apply nematodes with 200 ltr. of water/ha directly on the seeds. Although the insect larvae occur approximately a month later, the nematodes persist long enough to control the pest. Insects penetrate into the roots where they are not easily reached by insecticides, whereas nematodes follow the insects into the galleries and kill the larvae 2-3 days after infestation. Latest field results, which have used the novel application technology, will be presented. Since product costs reach almost the same level like chemical insecticides and since the seed treatment with neonicotinoides was banned by the European Commission in 2013, the product dianem® is in commercial use for the first time on larger scale against this invasive maize pest.

Contributed paper. Wednesday, 11:30 **150**

Evaluation of Ten Plant Extracts as Ultraviolet Protectants for *Spodoptera littoralis* nucleopolyhedrovirus

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Ten plant extracts were tested as ultraviolet protectants to improve the persistence of *Spodoptera littoralis* multiple embedded nucleopolyhedrovirus (SpliMNPV). In an initial test, the SpliMNPV alone or in combination with 10 plant extracts, each at a concentration of 1% was exposed to ultraviolet B (UV-B) for one hour. Among them, five plant extracts, viz. cloves, henna, green tea, pomegranate and grape showed a high rate of virus protection with original activity remaining

(OAR) at 100 %, 97 %, 91 %, 90.6 %, and 77 %, respectively. However, lemon, kiwi, olive, dates and beetroot extracts provided lower protection with OARs of 71 %, 58.4 %, 53 %, 21 %, and 18 %, respectively. Using the same UVB source, secondary screening was carried out on the five best additives from primary screen, and tested at a concentration of 0.5% and using an exposure timing of 5 hours. Clove and henna showed the highest rate of protection with OAR of 96.6% and 76.5%, respectively. In addition, absorption spectra and the obtained protection rate were correlated. These laboratory findings are very encouraging and that field studies are underway.

Contributed paper. Wednesday, 10:45 **151**

Interactions among Fungal and Viral Pathogens and Parasitoids

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The majority of studies of pathogens are conducted with one pathogen species or even strain and one host species, although in nature numerous pathogens and other parasites form a community attacking a host, and even attacking the same host individual. We conducted studies of natural enemy interactions using *Lymantria dispar* larvae, the fungal pathogen *Entomophaga maimaiga*, the viral pathogen *Ld*MNPV and parasitoids, all of which have been introduced to North America. Studies in low density populations were conducted in central New York State over 16 years and high density populations were studied at 59 sites in the mid-Atlantic region in 2009, when an outbreak population was crashing. We found very different interactions at low versus high host population densities. At low host density, *E. maimaiga* and parasitoids were both fairly abundant and *Ld*MNPV infections were almost nonexistent. At virtually all higher density sites the emergent *E. maimaiga* was most abundant. Virus infection was positively associated with host density while *E. maimaiga* and parasitoids were both frequency dependent. *E. maimaiga* and parasitoids co-occurred in the same larvae less than expected and *Ld*MNPV and parasitoids co-occurred in the same larvae more than expected while the fungus and virus reproduced in the same cadaver as expected, suggesting little interaction. This pattern of co-occurrence suggests that the two semelparous natural enemies (fungus and parasitoid) seldom successfully share a host larva while the iteroparous virus was more successful in co-inhabiting with either *E. maimaiga* or parasitoids.

Contributed paper. Wednesday, 12:00 **152**

Oryctes rhinoceros* population diversity and potential implications for control using *Oryctes nudivirus

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The discovery of *Oryctes nudivirus* (OrNV) in the 1960s by Dr Alois Huger enabled the successful management of coconut rhinoceros beetle (*Orytes rhinoceros*) populations through classical biocontrol release in the Pacific, SE Asian, and Indian Ocean regions. OrNV continues to be an important biocontrol agent for the control of *Oryctes rhinoceros* in both coconut and oil palm growing regions. Augmentative release of OrNV is commonly used to enhance the natural spread and ensure its continued presence within palm growing regions and surrounding areas. For over four decades after the distribution of the virus, *O. rhinoceros* was not reported to have established in any new regions. However, in 2007 the beetle was discovered in Guam and the population has now established with a highly damaging outbreak such as those not seen for 40+ years. Initial attempts to introduce OrNV into the Guam population were unexpectedly unsuccessful. This has raised the possibility the *O. rhinoceros* population that invaded Guam is less susceptible to OrNV, or potentially resistant. Furthermore, near the end of 2013, a population of *O. rhinoceros* was detected in Hawaii, although it is not believed to have established yet. The discovery of new *O. rhinoceros* invasions within the Pacific region linked with the possibility of a virus tolerant population suggests the beetle may again be on the move. To assist efforts in identifying the source populations for the Guam outbreak, a simple PCR-RFLP method has been developed to distinguish Guam *O. rhinoceros* from other populations. Analysis of several *O. rhinoceros* populations has demonstrated that the Hawaiian beetles are of the same haplotype as those found in Guam. We will discuss current results in relation to what is known about these new invasions and potential implications for the future.

Contributed paper. Wednesday, 12:15 **153**

The Control of Fungi Using with Liposomal Formulation of Essential Oil of *Satureja hortensis* and its cell viability assay

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Satureja hortensis is an annual herb used as nostrum in Eastern Anatolia region of Turkey for the treatment of different infectious diseases and disorders. It can also be utilized as a biopesticide against greenhouse pests. The aim of the present study was to control *Candida albicans* with liposomal formulation of essential oil of *S. hortensis* incorporated into an ointment. Also toxicity of the liposomal essential oil of *S. hortensis* was investigated by MTS assay analysis on L929 mouse fibroblast cell lines. The liposomal formulations were designed using thin film technique and liposomes were properly incorporated into the ointment. The chemical composition of the essential oil obtained from *S. hortensis* was determined by GC and GC-MS analysis. The liposomal essential oil of *S. hortensis* was tested against *Candida albicans* with disc diffusion assay and micro-well dilution assay. Then the toxicity of liposomal essential oil on mammalian cells was determined with MTS analysis. The results of antifungal tests showed that the essential oil of *S. hortensis* incorporated into the ointment and liposomal essential oil formulation have potential antifungal activity against *Candida albicans*. MTS assay results showed that a concentration of 10^{-7} % liposomal essential oil formulation is the safe dose for L929 mouse fibroblast cells. This liposomal formulation dramatically increases antifungal activity by improving cellular intake without side effects on mammalian cells.

VIRUSES 4

Contributed paper. Wednesday, 10:30 **154**

Mamestra configurata nucleopolyhedrovirus-A transcriptome from infected host midgut

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Infection of an insect by a baculovirus occurs in two distinct phases, an initial infection of host midgut by occlusion-derived virions (ODVs) and subsequent systemic infection of other tissues by budded virions (BV). A vast majority of investigations of the infection process have been restricted to cell culture studies using BV that emulate the systemic phase of infection. In the current study we investigate baculovirus gene expression in ODV infected midgut cells. We have focused on the critical first phase of in vivo infection by Mamestra configurata nucleopolyhedrovirus-A in *M. configurata* larvae, using qPCR and RNAseq mass sequencing strategies to examine virus gene expression in midgut cells. The earliest genes detected by each method had significant overlap and included known early baculovirus genes as well as genes unique to MacoNPV-A and genes of unknown function. The RNAseq datasets also revealed a large range of expression levels across most ORFs. These datasets provide a whole genome transcriptomic analysis of viral genes required for virus infection in vivo and will provide the basis for functionally analyzing specific genes that may be critical elements in baculovirus midgut infectivity and host range.

Contributed paper. Wednesday, 10:45 **155-STU**

Genomic adaptation to different hosts – Impact of genetic diversity on viral fitness

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Ecological and genomic adaptations underpin evolutionary processes. Nucleopolyhedroviruses, enclosing many virions in their occlusion bodies, evolve as populations of genomes, adapting to particular ecological niches. We previously showed that all the possible variation is present in a genome population of the size of baculoviruses. When adapting to a new niche, genome populations should differentiate. We conducted experimental evolution on AcMNPV wild type population by passaging 10 times through 4 different host species, in 10 replicates. We then characterised the genetic make up the original and evolved baculovirus populations by ultra-deep Illumina sequencing and their phenotypes by virulence bioassays. We were able to compare virulence components (time, dose and yield) to population diversity. Our experiment allowed us to follow the evolution of a population of genome and its phenotype in different environments to link fitness with genomic changes.
From all the evolved populations, different profiles emerge, with different relations between intra-population variation and fitness. Actually, it seems that all the species that have evolved on a host show a reduction of intra-population variation while increasing fitness on this host. But when looking at the generalist potential of the population, a lower diversity doesn't always bring a lower fitness. Of course, there are variations in these results that seem to be modulated with the primary fitness of the virus to the infected host; spectacular fitness increase can emerge when infecting a very resistant host. These results give new indications in the evolution of the relation between fitness and genetic diversity.

Contributed paper. Wednesday, 11:00 **156-STU**

Transcriptomic analysis of a host-parasitoid interaction between a Hymenoptera *Cotesia congregata*, a Lepidoptera *Manduca sexta* and a Polydnaviridae

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Cotesia congregata develops as a gregarious endoparasitoid into larvae of the tobacco hornworm *Manduca sexta*. The parasitoid wasp has evolved virulence strategies using an obligatory viral symbiont from the Polydnavirus (PDV) family named *Cotesia congregata bracovirus* (CcBV). CcBV particles are produced by specialized cells of the wasp ovaries and are injected along with the eggs into the host body and act by manipulating host immune defenses, and development, thereby enabling wasps to survive in a potentially harmful environment.
In the caterpillar host, the expression of only a few selected candidate virulence genes had been studied, and so far we lacked a global vision of viral and host gene expression.
To identify viral and host gene regulation during parasitism we performed a large-scale transcriptomic analysis by *454* sequencing of two distinct immune tissues (fat body and hemocytes) of the host *M. sexta* isolated in four experimental contexts: (i) non-treated *M. sexta*; (ii) parasitism of *M. sexta* by *C. congregata*; (iii) immune stimulation of *M. sexta* by heat-killed bacteria; (iv) parasitism of *M. sexta* by *C. congregata* followed by bacterial challenge. Following this analysis, we were able to identify 76 CcBV genes and 1993 *M. sexta* genes expressed 24hrs after parasitism.
The data obtained allows us to draw for the first time a functional map of the CcBV genome, and to visualize at a global level *M. sexta* genes that are regulated during parasitism. This type of analysis will help us to highlight viral virulence genes that play an essential role in the host-parasitoid interaction.

Contributed paper. Wednesday, 11:15 **157**

Expressed viral ORF and new virus discovery from high throughput transcriptomes of non-model animal

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High-throughput sequencing allows quantifying the viral biodiversity by studying the diversity of endogenous viral ORF and discovering new virus pathogens of impacting host species. A total of 114 non-model wild animal species from 33 taxonomic groups (i.e. 441 individual transcriptomes) were screened. Virus detection pipeline started with de-novo assembly of Illumina reads and prediction of 17 million ORF. Protein annotation was performed by a sequence homology search. Taxonomic assignment of each ORF was finally achieved using the NCBI taxonomy database.
Viral ORF from 8 species of termites, mosquito, ants, crustacean and marine annelid were analyzed thus far. We detected 146 viral ORF, i.e. 10 viral ORF per host species, mostly related to dsDNA viruses. Genomic analysis showed that their (G+C) content was at intermediate level between those from host genes and from exogenous viruses, suggesting a genuine and recent viral origin. Viral ORF were shorter than their exogenous counterparts but still expressed: their function might have thus been retained. This result illustrated potential cases of viral gene domestications by the host's genomes.
A dozen of complete viral genomes were identified thus far; mostly RNA viruses. Molecular phylogenies allowed assessing the taxonomic position of the viruses. *Lake sinai virus-like* (LSV; unclassified ssRNA virus) were discovered in ants and solitary bees. LSV was recently discovered in honey-bees associated with colony collapse disorder. LSV-like discovery in non-*Apis* insects suggests that hymenopterans could act as a viral reservoir toward domesticated bees. This work illustrated the great potential of our method for high-throughput virus discovery.

Contributed paper. Wednesday, 11:30 **158**

Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons

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Horizontal transfer (HT) of DNA is an important factor shaping eukaryote evolution. Although several hundreds of eukaryote-to-eukaryote HTs of transposable elements (TEs) have been reported, the vectors underlying these transfers remain elusive. Here, we show that multiple copies of two TEs from the cabbage looper (*Trichoplusia ni*) transposed in vivo into genomes of the baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) during caterpillar infection. We further demonstrate that both TEs underwent recent HT between several sympatric moth species (*T. ni*, *Manduca sexta*, *Helicoverpa spp.*) showing different degrees of susceptibility to AcMNPV. Based on two independent population genomics data sets (reaching a total coverage >330,000X), we report a frequency of one moth TE in 8,500 AcMNPV genomes. Together, our results provide strong support for the role of viruses as vectors of TE HT between animals, and they call for a systematic evaluation of the frequency and impact of virus-mediated HT on the evolution of host genomes.

Contributed paper. Wednesday, 11:45 **159**

Genomic analysis of five *Lymantria dispar multiple nucleopolyhedrovirus* isolates and biological activity against different host strains of *Lymantria dispar*

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To evaluate genetic diversity of *Lymantria dispar multiple nucleopolyhedrovirus* (LdMNPV) at the genomic level, five isolates of LdMNPV from North America, Europe, and Asia were selected for complete genome sequence determination. These isolates consist of LdMNPV-2161 from Korea; LdMNPV-3029, a sample of the product Virin-Ensh, from Russia; LdMNPV-3041 from Japan; LdMNPV-3054 from Spain, and LdMNPV-Ab-a624, a plaque isolate from a sample collected in Massachussetts, USA. The genome sequences of these isolates were co-linear with the genome sequence of the reference isolate LdMNPV 5-6, derived from the Gypchek product. LdMNPV 5-6 ORFs ld31, ld66, and ld133 were not found in the other five isolates, while all other ORFs annotated for isolate 5-6 were present in at least one other isolate. The greatest degree of sequence divergence among the isolates was observed among the *bro* genes, especially in the two clusters of *bro* genes between *chitinase* (ld70) and ld76 and between ld111 and *dutpase* (ld116). A 2-nt deletion in the enhancin gene *vef-2* (ld160) of LdMNPV-Ab-a624 resulted in a frameshift and truncation of the *vef-2* ORF, while a deletion in LdMNPV-3041 entirely removed *vef-1* (ld65). Bioassays against the New Jersey Standard Strain of *L. dispar* did not indicate any reduced pathogenicity due to mutation or deletion of *vef* genes in either isolate 3041 or Ab-a624. In bioassays against *L. dispar* from Japan, Russia, Europe, and North America, isolates 2161, 3029, and 3041 exhibited a greater degree of pathogenicity against neonate larvae than a sample of Gypchek at the lower dose range.

Contributed paper. Wednesday, 12:00 **160**

Phylogenomics reveals ecological factors that lead to speciation in *Baculoviridae*

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The study of drivers of species diversification is complex due to the entanglement of numerous ecological factors defining ecological niches. By their nature, viruses provide a confined system of interactions to study diversification from micro to macroevolution. Virus ecological niches are clearly defined by their hosts, which consequently should primarily drive virus evolution. Baculoviruses (BVs) have been well studied, showing the peculiarity of BV life cycle with the dissemination of viral particles on insect host plants. This strongly suggests that host plants may play an important role in their evolution. Here we study phylogenetic patterns of host use in the large radiation of BVs that attack the insect order Lepidoptera (moths and butterflies). We generated a phylogeny for ~500

BV isolates using four core genes (*polh*, *lef-8*, *lef-9*, *pif-2*) from which we delimited virus species, to obtain a comprehensive timed molecular BV species phylogeny. We then used a combination of phylogenetic (BV and insects) and ecological (host range of BVs, host plant range of lepidopteran hosts) data to address the following hypothesis: BVs are host specialists and show high levels of phylogenetic conservatism, BVs have the same ages as their lepidopteran hosts and the host plants of the insects drive also the evolution of BVs. We found that in general, hosts primarily induced BV species speciation over a short timeframe. But on a larger evolutionary scale, the insect-host co-evolutionary relationship signal is confused. Surprisingly we revealed that insect host plant specificity contributed largely to BV evolutionary history.

CONTRIBUTED PAPERS Wednesday, 10:30-12:15

FUNGI 5

Contributed paper. Wednesday, 10:30 **162**

An entomopathogenic strain of *Beauveria bassiana* against *Frankliniella occidentalis* with no detrimental effect on the predatory mite *Neoseiulus barkeri*

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Among 28 isolates of *Beauveria bassiana* tested for virulence against *F. occidentalis* in laboratory bioassays, we found strain SZ-26 as the most potent, causing 96% mortality in adults at 1×10^7 mL^{-1}conidia after 4 days. The effect of the strain SZ-26 on survival, longevity and fecundity of the predatory mite *Neoseiulus* (*Amblyseius*) *barkeri* Hughes were studied under laboratory conditions. The bioassay results showed that the corrected mortalities were less than 4 and 8% at 10 days following inoculation of the adult and the larvae of the predator, respectively, with 1×10^7 conidia mL^{-1} of SZ-26. Furthermore, no fungal hyphae were found in dead predators. The oviposition and postoviposition durations, longevity, and fecundity displayed no significant differences after inoculation with SZ-26 using first-instar larvae of *F. occidentalis* as prey in comparison with untreated predator. In contrast, the preoviposition durations were significantly longer. Observations with a scanning electron microscope, revealed that many conidia were attached to the cuticles of *F. occidentalis* at 2 h after treatment with germ tubes oriented toward cuticle at 24 h, penetration of the insect cuticle at 36 h, and finally, fungal colonization of the whole insect body at 60 h. In contrast, we never observed penetration of the predator's cuticle and conidia were shed gradually from the body, further demonstrating that *B. bassiana* strain SZ-26 show high toxicity against *F. occidentalis* but no pathogenicity to predatory mite.

Contributed paper. Wednesday, 10:45 **163-STU**

Interactions between the insect pathogenic fungus *Metarhizium*, the wheat pathogen *Fusarium culmorum* and the mycoparasitic fungus *Clonostachys rosea*

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The current study was conducted to determine if wheat seeds co-inoculated with the insect-pathogenic fungus *Metarhizium* (three species) and the mycoparasitic fungus *Clonostachys rosea* are protected from both insect pests and plant pathogens. The experiment was done in two parts: First, a co-infection bioassay was performed to determine if the virulence of *Metarhizium* was affected by the presence of other fungi by co-treating *Tenebrio molitor* larvae with combinations of *Metarhizium*, *C. rosea*, and the wheat pathogen *Fusarium culmorum*. Second, wheat seeds were co-inoculated with the both beneficial fungi and compared to single inoculations of the effects on *F. culmorum* when allowed to grow for two weeks under controlled laboratory conditions. The resulting root systems were then placed with *T. molitor* larvae which were evaluated daily for mortality. Pathogenicity to insect persisted in all treatments, but *Metarhizium* virulence was affected by co-treatments with other fungi. Root-infection by *F. culmorum* was not reduced directly by the presence of *Metarhizium* while *C. rosea* reduced *F. culmorum* infection and this effect was not diminished in combination with *Metarhizium*. The results of this study suggest that combination of beneficial fungi may effectively protect roots from both pathogens and insects pests..

Contributed paper. Wednesday, 11:00 **164**

Diversity, ecology and virulence of entomopathogenic fungi isolates naturally infecting the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the Mediterranean Basin

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), and the moth *Paysandisia archon* (Burmeister) (Lepidoptera: Castniidae) are considered nowadays the most important palm pest worldwide. Current tactics commonly used to manage the weevil are based on chemical control, although the use of these compounds is hampered by several environmental concerns. In recent years, the *R. ferrugineus* (Rf) microbial control potential of entomopathogenic fungi (EPF) has been highlighted.
In this work, several strains of EPF have been isolated from diverse naturally infected specimens of both species, found in different countries through the Mediterranean Basin.
Firstly, the usefulness of the elongation factor 1-alpha (EF1-α) region, the nuclear intergenic region BLOC and inter simple sequence repeat (ISSR) or microsatellite markers were assessed as *R. ferrugineus* EPFs diagnostic tool, alone or in combination, and relationships among the Mediterranean *Beauveria* and *Metarhizium* isolates obtained from the red palm weevil were inferred.
Secondly, the effect of diverse environmental parameters such as temperature, humidity and UV-B radiation were assessed on germination and colony growth of these EPFs strains as function of their genealogy and geographic origin.
Finally, virulence of selected isolates was tested against both Rf larvae and adults.
Our results show a distribution pattern of *Beauveria bassiana* through the Mediterranean Basin, possibly associated with the host insect dispersion, with the same genetic group presented throughout the European distribution area of phytophagous. Furthermore, several differences were observed between the different genetic groups found, regarding the different factors analyzed: temperature, humidity, UV-B radiation and virulence.

Contributed paper. Wednesday, 11:15 **165_STU**

Recovery and detection of an entomopathogenic endophyte: overcoming the challenges involved

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The prospect of utilising entomopathogenic fungi, such as *Beauveria* spp., as endophytes to enhance their biological control activity is presently a highly topical area of research. However, the endophyte-host relationship is complex and the associated methodology for defining and recovering endophytic strains wrought with problems. A review of the literature on endophyte isolation methods indicated the need for developing a methodology for reliable molecular detection of *Beauveria* spp. *in planta*. The method that was developed included a stringent surface sodium hypochlorite and ethanol based sterilisation with the protocol optimized specifically for propagules of *Beauveria* spp.. This was followed by treatment of plant tissue with propidium monoazide (PMA^{TM}) to exclude surface DNA contamination from subsequent PCR. A nested PCR/RT-qPCR protocol capable of detecting as little as 32 fg was developed using novel primer sets designed from the translation elongation factor 1-α gene (TEF). Additionally, epiphytic DNA was isolated separately using a benzyl chloride treatment in order to determine any corresponding occurrence of *Beauveria* with endophyte positive samples. Freshly inoculated samples of *Zea mays* and *Solanum lycopersicum* were surface sterilized using the optimized method and various controls were included for comparison to determine at which stage(s) *B. bassiana* remained viable. Results suggest that *Beauveria* DNA contamination and viability after surface sterilisation is a common and confounding issue associated with endophyte detection and isolation. However, this may be overcome with the improved methodology described here, which delivers reliable detection of endophytic strains.

Contributed paper. Wednesday, 11:30 **166-STU**

Intense spatio temporal pattern in pathogen-host interaction between *Pandora formicae* and *Formica rufa*

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Pandora formicae (*Entomophthorales*, Entomophthoromycota) is a pathogen of the common red wood ant (*Formica rufa*), causing symptoms of "summit disease", where ants attacked by the fungus place themselves on an elevated position before death and sporulation, enabling the pathogen to release infectious spores widely. This facilitates fungal transmission but puts the ant colonies at an enhanced risk of a lethal disease outbreak. Ant workers, on the other hand, respond by removing the cadavers from the nest surroundings, by that lowering the load of conidia, but at the same time putting them at risk while protecting the colony from this hazard. Detailed mapping of the cadavers around an ant nest, twice a day for three subsequent days, three times during one season, shows how ants' behavioral response keeps the fungus prevalence 'at hold'. It also shows the uniqueness of this interaction, the only known example of an entomophthoralean fungus infecting a social insect host, and an evolutionary adjustment of fungal life strategy to maintain itself in the host population without causing rapid epidemics.

Contributed paper. Wednesday, 11:45 **167**

Patterns of host adaptation in fly infecting *Entomophthora* species

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Insect pathogenic fungi (IPF) differ widely in their capability to infect different hosts. Some are generalists and will, given a sufficient number of infectious spores are present, infect almost any species of insect (e.g. Hypocrealean *Metarhizium* and *Beauveria*). Members of a different main IPF phylum Entomophthoromycota generally have more narrow host-ranges where some species for example only infect aphids or only locusts. Certain species (or strains) are even more host specific and are only known to infect a single or very few taxonomically related insect species under natural conditions (e.g. *Entomophthora*, *Strongwellsea* and *Entomophaga*). Species diversification of the obligate IPF within Entomophthoromycota thus seems to be primarily driven by co-evolutionary host adaptation to specific insect families, genera or species-complexes, but the underlying genetic factors of host adaptation in this fungal order are largely unknown and leave many unanswered questions. For example are the numbers of virulence factors increasing, or decreasing when fungal pathogens adapt to a narrow range of potential hosts? And, are host specialization based on many genetic changes with small effect or few with large effect? Here we examine closely related species within the *Entomophthora muscae* species complex: *E. muscae s. str.* infecting the common housefly *Musca domestica* and *E. muscae* s.l. strains infecting the cabbage fly *Delia radicum.* We use RNA-seq based comparative transcriptomics to unravel genetic differences and similarities in order to detect patterns of host-specific molecular adaptation.

Contributed paper. Wednesday, 12:00 **168-STU**

Plant volatile organic compound manipulation by endophytic entomopathogenic fungi

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The potential of entomopathogenic fungi (EPF) to live as endophytes in various plant tissues has been demonstrated several times in recent years. However, the effects of plant colonization by these endophytes on the metabolism of the colonized plants have been only rarely addressed. We analyzed the volatile organic compound (VOC) profiles of tomato plants (*Solanum lycopersicon*) inoculated with three strains of *Beauveria bassiana* and a plant pathogen biocontrol agent (*Trichoderma koningiopsis*) compared to control plants. We hypothesized that EPF colonized plants should be more attractive for herbivores, mediated by the VOC profiles, but should not exhibit differences when colonized by the plant pathogen antagonist. We found that *B. bassiana* and *T. koningiopsis* inoculated plants had significantly modified VOC profiles, with marked differences between different isolates. Some of the compounds up- or down-regulated are known to play a role in plant-herbivore interactions such as α-pinene, ß-

cymene, α-Terpinolen, ß-Phellandrene, Caryophyllene and α-Caryophyllene. When aphids (*M. persicae*) were allowed to colonize these plants, VOC profiles again differed with regard to specific compounds and the amount produced. However aphids did not discriminate between tomato plants inoculated with different endophyte isolates compared to control plants. We speculate that the VOC pattern found may play a role for attraction of natural enemies (parasitoids), competing with the EPF for the herbivores.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

MICROSPORIDIA 1

Contributed paper. Wednesday, 14:00 **169**

Effects of the microsporidium *Nosema adaliae* on the multicoloured Asian lady beetle, *Harmonia axyridis*

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Originally imported for use as a biological control agent for pest insects, the multicoloured Asian lady beetle, *Harmonia axyridis* Pallas has itself become a pest in many areas of the world. While it is very effective for biological control, *H. axyridis* tends to displace many native lady beetle species and the alkaloids produced by these beetles may affect the palatability of wine and have adverse effects on human health. The geographic distribution of *H. axyridis* extends throughout North America into Nova Scotia and overlaps with the range of the native two-spotted lady beetle, *Adalia bipunctata* L. The microsporidium *Nosema adaliae* was recently found in a native population of *A. bipunctata* from Nova Scotia and the geographic overlap of *A. bipunctata* with *H., axyridis* provides the opportunity for this microsporidium to be transmitted horizontally to *H. axyridis* in nature. In this study, *H. axyridis* larvae were provided with a combination of uninfected and *N. adaliae*-infected eggs. All of the *H. axyridis* larvae that consumed *N. adaliae*-infected eggs became infected with the pathogen. *H. axyridis* larval development was prolonged significantly, depending on the number of eggs eaten. These results suggest that there is potential for *N. adaliae* to be transmitted to *H. axyridis* in nature if the larvae consume a sufficient number of microsporidia-infected eggs.)

Contributed paper. Wednesday, 14:15 **170-STU**

Effects of two microsporidia from lady beetles on the green lacewing, *Chrysoperla carnea*

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The larvae of green lacewings, *Chrysoperla carnea* (Stephens), are generalist predators that feed on insect eggs, small caterpillars and other, soft-bodied insects. Lacewing larvae are commercially available for aphid control on various agricultural crops. It is common to use several types of biological control agents for controlling aphids at a given time to optimize pest control. Two-spotted lady beetles, *Adalia bipuncata* L., and convergent lady beetles, *Hippodamia convergens* Guerin-Meneville, are often released for aphid control in the same areas that lacewings are used. Two microsporidian pathogens infect these lady beetle species. Because lady beetles and green lacewings are often used simultaneously for aphid control, it is possible for lacewing larvae to become infected with microsporidia when infected eggs are eaten. The main objective of this study is to determine if microsporidia from lady beetles (*T. hippodamiae* and *N. adaliae*) are transmitted to green lacewings and to examine the effects of these pathogens on lacewing larvae and adults.

Contributed paper. Wednesday, 14:30 **171**

Features of the genomes of microsporidia in mosquitoes: status and preliminary findings

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The status and preliminary findings for full genome sequencing of two distantly related 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. *Edhazardia aedis* is the type species for the genus and has a limited host range in mosquitoes but 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 and transcriptome sequencing for *E. aedis* and *V. culicis floridensis* is completed. *V. culicis floridensis* has a genome size of approximately 6.1Mb while *E. aedis* is nearly an order of magnitude larger at approximately 51.3Mb, yet the gene content difference is smaller, with 2,773 and 4,190 predicted genes in *V. culicis* and *E. aedis* respectively. RNA-seq data has been analyzed for multiple time points in the life cycle of each species to validate predicted gene structures and to examine gene expression. Preliminary analysis of genome evolution and differential gene expression between life cycle stages will be presented.

Contributed paper. Wednesday, 14:45 **172**

Multi-gene phylogeny applied to the taxonomy of microsporidian parasites of crustacean hosts

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*Hepatospor*a, a recently erected genus, infects epithelial cells of the hepatopancreas of decapod crustaceans. We isolated *Hepatospora* sp. from three different crustacean hosts, inhabiting different habitats and niches; marine edible crab (*Cancer pagurus*), estuarine and freshwater Chinese mitten crab (*Eriocheir sinensis*), and the marine mussel symbiont pea crab, (*Pinnotheres piscum*). Isolates were initially compared using histology and electron microscopy revealing variation in size, polar filament arrangement and nuclear development.

Despite these morphological differences, sequence analysis of the partial SSU rDNA gene did not provide the resolution for distinguishing the isolates (>99% similarity). To investigate relationships between isolates, purified spore samples from the parasite infecting *E. sinensis,* and *C. pagurus,* were prepared for Illumina sequencing. Six additional gene sequences were mined from the resulting genomic data (RNA polymerase, Arginyl tRNA synthetase, Prolyl tRNA synthetase, Chitin synthase, Beta Tubulin and Heat Shock Protein 70). Primers were designed based on the above gene sequences to compare isolates, and to assess corresponding sequences in the genome of the isolate infecting pea crabs. Concatenated phylogenies using sequence data from the six genes revealed that *Hepatospora* isolates from the three different crustacean hosts are likely to be a single species. As such, the concatenated phylogeny supported that derived from analysis of SSU rDNA. Given the host, ecological and morphological distinction of the parasites infecting these three crabs, we provide further support for the concept that morphology is an inappropriate discriminator for even closely related taxa within the phylum, *Hepatospora* may form a widely distributed parasitic 'cline' within the hepatopancreas of aquatic crustacean hosts.

Contributed paper. Wednesday, 15:00 **173-STU**

Understanding the evolutionary loss of glycolysis in intranuclear crab microsporidians

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Enterocytozoon bieneusi is responsible for most cases of microsporidiosis in humans. Interestingly, an intranuclear microsporidian recently isolated from edible crabs appears to be a close relative of this human parasite on SSU based phylogenetic trees. Since microsporidians are known to be devoid of functional mitochondria and rely solely on glycolysis and energy import from their hosts, it was interesting to find that genes coding for glycolytic enzymes were absent from the genome of both parasites. Also, more recent genomic analyses of *Hepatospora spp.,* another crustacean parasite show a similar loss of glycolytic enzymes alluding to a single loss of glycolytic capabilities prior to the divergence of the Enterocytozoonidae lineage.
Absence of glycolysis may be compensated by the increase in host-ATP availability created by the aggregation of host mitochondria around microsporidian meronts, a feature often observed in microsporidian infections. However, *E. canceri* is an intranuclear parasite and hence, physically walled off from the host mitochondria. This may highlight the presence of a novel host nuclei-dependent metabolic process. To this end, phylogenetic and structural domain analyses on the first enzyme of the glycolytic pathway, hexokinase has revealed that it is severely mutated in deep branching microsporidia hinting to a different substrate-specificity adaptation. Most intriguing is the adjunction of a PTPA domain on the hexokinase of *E. canceri*. This is the first time severe mutations of hexokinase conserved domains have been documented in eukaryotic cells and our current efforts are directed towards understanding whether these mutations are associated with loss of enzyme function.

Contributed paper. Wednesday, 15:15 **174-STU**

Temporal trends and the effect of seasonal temperature on the prevalence of *Nosema* spp. in *Apis mellifera* in north-east Germany

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Apis mellifera (European honey bee) has long been host to *Nosema apis* and *Apis ceranae* (Asian honey bee) to *Nosema ceranae*. Natural infections of *N. ceranae* were first discovered in *A. mellifera* colonies in 2005. *N. ceranae* has already replaced *N. apis* in the warmer European countries and. as an emergent pathogen, is very competitive. It has been found to be more better adapted to higher temperatures than *N. apis* and is considered more virulent than *N. apis*. Lab experiments have shown the differential effect of temperature on *N. apis* and *N. ceranae* with respect to spore germination and virulence of the pathogen within the host.
Our study is based on a 10-year (2005-2014) cohort study of roughly 20 apiaries from north-east Germany, monitored in autumn and spring. Trend analyses show a significant increase in the prevalence of *N. ceranae* and a decrease in that of *N. apis,* suggesting the gradual replacement of *N. apis* by *N. ceranae.* Weather aggregates from different periods of the season preceding the colony sampling were tested against the proportion of infected colonies. They reveal considerable variability in their effects on *Nosema* prevalence. We are able to confirm, for the first time, the effect of seasonal temperatures on the prevalence of *Nosema* in honey bee colonies. Effect of North Atlantic Oscillation (NAO) indices were also tested as proxies for seasonal weather and were found to be reasonably good predictors of *Nosema* prevalence.

Contributed paper. Wednesday, 15:30 **175-STU**

Characterising putative virulence factors of the bee pathogen *Nosema ceranae*

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Microsporidia are obligate intracellular eukaryotic parasites related to fungi, possessing greatly reduced genomic and cellular components. The microsporidian *Nosema ceranae* threatens the two economically important pollinators, honey (*Apis mellifera*) and bumble (*Bombus* species) bees and has been causally linked to colony collapse disorder. Nosemosis has a complex epidemiology affected by host, pathogen and environmental factors. Although a draft of the *N. ceranae* genome has been published, the molecular basis underpinning pathogenicity is not known. The lack of established culturing techniques and a tractable genetic system necessitates use of model systems for both host and parasite such as *Saccharomyces cerevisiae*. We hypothesis effectors essential to disease progression exist amongst *N. ceranae* secretome genes. In this study we have started characterising these genes using Gateway® cloning technology and identify candidate effectors by their expression in *S. cerevisiae*. We offer experimental data supporting the identities of NcORF-01664 and NcORF-01663 as polar tube proteins (PTP) 1 and 2

respectively and identify a putative PTP4 through their capacity to induce morphological deformities in *S. cerevisiae.* We also show two unknown proteins are targeted to lipid droplets which could function to mobilise resources from this energy-rich organelle. In the future we hope to confirm this function is retained in a system more closely related to the insect host tissue using the *D. melanogaster* Gal4/UAS method. Increased knowledge on virulence factors and disease progression will ultimately lead to disease mitigation.

Contributed paper. Wednesday, 15:45 **176**

Detection of Microsporidia in Gammarids in the Delta of the Kuban River (Azov Sea, Russia)

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Gammarids of the Kuban river basin were surveyed for microsporidia infections at two sites: a) Azov sea coast close to the river Protoka mouth and b) a quarry lake 11 km eastwards from the sea. At the first site, a population of *Dikerogammarus villosus* was abundant in the intertidal zone. In June, 5 out of 100 specimens displayed developed infections with a monomorphic microsporidium. Its ribosomal RNA gene sequence showed high (above 99%) similarity to *Anncaliia algerae* with no variability between isolates from individual hosts. In July, the microsporidia were absent in gammarids (N=100). At the second site, in the quarry lake and neighboring ditches, there was an abundant population of *Gammarus* sp. infected with a dimorphic microsporidium at the rates of 100% in May and 50-80% in June. Sequencing of four cloned SSU rRNA gene amplicons (ca 900 bp long) from an individual host sample produced four distinct (97.8-99.4% similarity) haplotypes, suggesting infection with multiple genetically distinct isolates or species of genus *Dyctiocoela*. The latter taxon unites common and widespread gammarid-infecting microsporidia and revealing a new species of *Dyctiocoela* in these hosts is quite expected. Conversely, the detection of an *A. algerae*-like parasite in gammarids is somewhat unusual, though logical given the broad host range of *A. algerae* and its ability to develop in amphipods upon injection of spores into the hemocoel. This pathogen has potential risk for human infection and should be taken into account when considering safety of public beaches. Supported by RFBR, 13-04-00284 and 14-04-91176.

CONTRIBUTED PAPERS Wednesday, 14:15-15:45

MICROBIAL CONTROL 3

Contributed paper. Wednesday, 14:15 **178-STU**

Synthesis and Characterization of fungus mediated silver nanoparticle for the toxicity on filarial Vector, *Culex quinquefasciatus*

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Larvicidal activities on myco-synthesized silver nano-particles (AgNPs) against filarial vector, *Cx. quinquefasciatus*. The AgNPs synthesized by filamentous fungus, *Penicillium verrucosum*. Characterized by UV-Vis spectrophotometer, Fourier transform infrared spectroscopy, scanning electron microscopy, and transmission electron microscopy. Furthermore, laboratory evaluation of fungus mediated silver nano-particle against larvae and pupae of *Cx.quinquefasciatus*. The characterization studies confirmed the spherical shape and size (3–24 nm) of silver nano-particles. The efficacy of fungus AgNPs tested concentrations of 25 and 50 ppm against L1, L2, L3 and L4 instar larvae of *Cx. Quinquefasciatus. The* LC_{50} (LC_{90}) values are 4.91 (8.13), 5.16 (8.44), 5.95 (7.76) and 7.83 (12.63) in L1 to L4 instar at 25 ppm. Whereas, LC_{50} (LC_{90}) were 5.24 (8.66), 5.56 (8.85), 6.20 (10.01) and 7.04 (10.92) in L1 to L4 instars treated at 50 ppm. The mortality rates were positively correlated with the concentration of AgNPs. Significant ($P<0.05$) changes in the larval mortality was also recorded between the period of exposure against all instar of larvae of *Cx. quinquefasciatus*. These finding use of fungus synthesize silver nano-particles is a rapid, eco-friendly, and a single-step approach and potential mosquito larvicidal agents.

Contributed paper. Wednesday, 14:30 **179-STU**

Entomopathogenic fungi as endophytes: interaction with phytohormones

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With increasing interest in entomopathogenic fungi as endophytes (EEPF) in biological control strategies, there is a need for more background information on the interaction of these fungi with their host plant metabolism. Several studies have already reported on changes in the dry weight of plants when endophytically colonized by these EEPFs; however, a more detailed understanding of plant-fungus-interactions is missing. We measured phytohormone levels in plants with regard to the hypotheses that i) EEPFs produce phytohormones as fungal secondary metabolites when growing within plant tissues or ii) that plants react to the presence of EEPFs by increasing/decreasing their phytohormone production. We inoculated the seeds of tomato (*Solanum lycopersicum*) and cotton *(Gossypium hirsutum*) plants with one strain of *Beauveria bassiana* and three different strains of *Metarhizium anisopliae*, and grew these plants under standardized conditions in the greenhouse. We used LC-MS to analyse several phytohormones (including Salicylic Acid (SA), Abscisic Acid (ABA), Indolic Acetic Acid (IAA), Salicylic Acid Glucoside (SAG), and Jasmonic Acid (JA)) in eight weeks old leaves of these plants. The results will be discussed with regard to induced plant responses as well with regard to potential influences on herbivore-plant-interactions.

Contributed paper. Wednesday, 14:45 **180**

Pathogenicity of three entomopathogenic fungi on larvae and adults of the sisal weevil: The less the better?

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The sisal weevil was first recorded in Greece on May 2010 on ornamental plants of *Agave sp*. As the use of synthetic insecticides is prohibited in urban landscape areas the evaluation of potential biological control agents (BCAs) is essential. Indigenous strains of *Isaria fumosorosea* and *Metarhizium anisopliae* isolated from the soil and a strain of *Beauveria bassiana* obtained from a *Rhynchophorus ferrugineus* cadaver were chosen for evaluation.
Infection of adults and larvae of *S. acupunctatus* was achieved by immersing individuals in aqueous conidial suspensions. Additionally, natural diet of insects was also immersed in conidial suspensions and provided to individuals, in order to assess effectivity of application through treated surface. *Beauveria bassiana* and *M. anisopliae* were applied in concentrations of 10^7 and 10^6conidia/ml while *I. fumosorosea* was applied at a concentration of 10^6 conidia/ml. Mortality was recorded daily for up to 11 or 21 days for larvae and adults respectively. The highest adult mortality was achieved by *B. bassiana* through contact application reaching 100%, followed by *M. anisopliae* (48±10% to 28±10%) and *I. fumosorosea* (40±6.3%). In terms of larvae, mortality in all bioassays reached 100% with the exception of the treatment of contaminated diet by *I. fumosorosea* conidia (20±11%). All cadavers produced visible mycelium on their surface within a week. Results indicate a high level of mortality at the most harmful life stage of the pest, even at low concentrations and a lower level of mortality at the mobile adult stage. Benefits of a low concentration application of fungi are discussed..

Contributed paper. Wednesday, 15:00 **181**

Understanding *Beauveria bassiana* infection within its host *Triatoma infestans*: time course expression of genes encoding fungal toxic nonribosomal peptides and insect humoral immune proteins

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During invasion into insect hemocoel, some entomopathogenic fungi secrete toxins contributing to a successful infection. In response, insect cellular and humoral immune reactions are triggered. In this work, we studied by real-time PCR the expression pattern of *B. bassiana* genes involved in beauvericin, bassianolide, and tenellin biosynthesis throughout the infection process in nymphs of the kissing bug *T. infestans*. We also investigated the expression level of some bug proteins involved in the humoral immune response, i.e. prophenoloxidase, hemolectin and defensin. In conidia-treated insects, the expression of beauvericin synthetase, bassianolide synthetase, and tenellin synthetase peaked 6 days post-inoculation. In blastospore-injected bugs (bypassing the insect cuticle) the expression level peaked 12 hours post-injection. Regarding insect immune response, conidia treatment induced higher expression of defensin and hemolectin, with values of 8.3 ± 1.1 and 2.7 ± 1.4 fold inductions, respectively. In blastospore-treatment, the expression level of all genes tested raised from 12 to 48 hours, reaching 9.3 ± 3.6 (prophenoloxidase) and 26.6 ± 5.4 (defensin) fold induction. These results help to understand at the molecular level the “arm race” taking place in insect hemocoel during fungal invasion.

Contributed paper. Wednesday, 15:15 **182**

Compatibility of herbicides used in olive orchards with a *Metarhizium brunneum* strain used for the control of the olive fly preimaginals in the soil

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In vitro and *in vivo* studies were developed to evaluate the compatibility of the six most common herbicides applied to the soil of olive orchards with *Metarhizium brunneum* EAMa 01/58-Su strain against medfly *Ceratitis capitata* pre-imaginals. The fungus demonstrated high *in vitro* compatibility with the six active ingredients in malt agar medium, with growth rates (a) ranging between 2.5 mm d^{-1} and (Glyphosate) and 3.3 mm d^{-1} (Oxyfluorfen). This compatibility was also revealed *in vivo* by assaying the fungus towards medfly prepuratiating larvae in herbicide containing soil (at 1.0 x 10^8 conidia g $soil^{-1}$). Even if there was a decrease of the *M. brunneum* level until 10^4-10^5 conidia ml^{-1} in the soil 15 days after inoculation, mortality rates, which were in the range of 70-80%, did not differ significantly to the controls, except the ones observed in soils treated Glyphosate and its herbicide combinations, in which a significant 50% reduction of virulence was detected. These results reveal a general compatibility of *M. brunneum* with the most common herbicides applied to the soil of olive orchards, whereas a mixture of the fungus in the tank of the atomizer for a simultaneous treatment beneath the tree canopy is recommend for all active ingredients except Glyphosate.

Contributed paper. Wednesday, 15:30 **183**

The Seed Corn Maggot and *Metarhizium* are Related to Maize Yield in an Organic, Cover Crop-Based Farming Systems Experiment

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Organic farmers must largely rely on cultural practices and biological processes to prevent crop damage from pests. Many farmers are interested in using cover crop mixtures to gain production and ecosystem benefits, but there has been little research on the effects of cover crop diversity on arthropod pests and their natural enemies. The seed corn maggot, *Delia platura* (Diptera: Anthomyiidae), is an early-season pest of large-seeded crops in conventionally tilled systems. Insect-pathogenic fungi in the genus *Metarhizium* commonly occur in agricultural soils and infect soil-dwelling arthropods. In 2013, we examined the effects of overwintering cover crop diversity, ranging from one to 7 species, on seed corn maggot fly emergence, *Metarhizium* detection, soil characteristics, and corn yield. Seed corn maggot was detected in post-plant emergence traps from all treatments in maize and soybean, with approximately 10 times greater numbers captured from maize compared to soybean. Numbers of flies captured were not related to level of cover crop diversity. *Metarhizium* was detected in all treatments, with similar average detection rates in maize and soybean. Detection of *Metarhizium* was not related to level of cover crop diversity. In multivariate analyses, numbers of emerged flies relates negatively to maize yield and detection of *Metarhizium. Metarhizium* detection relates positively to maize yield, soil organic matter, electrical conductivity, and Mg. The negative relationship between emergence of seed corn maggot flies and *Metarhizium* suggests that this fungus is a natural mortality factor for seed corn maggot at this site.

VIRUSES 5

Contributed paper. Wednesday, 14:00 **184**

Soybean aphid viruses exploit contrasting transmission strategies

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The soybean aphid, *Aphis glycines* Matsumura, is an invasive pest of primary agricultural importance in North America. Following its introduction in 2000, soybean yields dropped 11%, with the costs of management and yield loss estimated to be $1.6 billion over a 10 year period. Soybean aphids are managed primarily by application of chemical insecticides. We identified two viruses from the soybean aphid transcriptome and small RNA data that may have potential for use in soybean aphid management: Aphid lethal paralysis virus (ALPV)-Ames (Dicistroviridae) and Aphis glycines virus (AGV; unclassified). There is evidence for the presence of ALPV-like viruses in several different insects including the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus), the honeybee *Apis mellifera*, and Western corn rootworm *Diabrotica virgifera virgifera*. AGV has a ~5 kb single stranded RNA (ssRNA) genome and forms a 30 nm particle. The RNA-dependent RNA polymerase (RdRp) of this virus is closely related to that of *Euprosterna elaeasa* virus (Tetraviridae), while the AGV coat protein (CP) is similar to those of plant *Sobemoviruses*. Based on RT-PCR of AGV RdRP sequence, AGV-like viruses appear to be present in two other aphid species, the bird cherry-oat aphid, *R. padi* and the green peach aphid, *Myzus persicae* (Sulzer). Notably, ALPV-Ames does not appear to be vertically transmitted in the soybean aphid, while AGV is 100% vertically transmitted. The different transmission strategies of these two viruses and the implications of 100% vertical transmission will be discussed.

Contributed paper. Wednesday, 14:15 **185**

Characterization of mechanisms involved in the transmission of a lepidopteran densovirus

Cécilia Multeau[1], Doriane Mutuel [2], Manuela Rakotomanga[2], Anne Kenaghan[2], Clément Bousquet[2], Rémy Froissart[3-4], Nathalie Volkoff[2] and Mylène Ogliastro[2]
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Densoviruses are small insect parvoviruses infectious per ingestion for several lepidopteran species. Their potential as microbial control agents led us to focus on the understanding of motors driving the transmission of densoviruses in the environment. Natural dynamic of densoviral infections was never investigated although several metagenomic studies revealed the presence of densoviruses in samples from various origins (feces from bats, mosquitoes, marine samples like urchins…). In this study, we qualitatively and quantitatively characterized direct and indirect mechanisms leading to the transmission of a model viral species, Junonia coenia densovirus, on the model host Spodoptera frugiperda. We showed that cannibalism of infected individuals and bites between infected and uninfected individuals are major events in the transmission of JcDNV while exposition to contaminated feces and/or regurgitations contributes to widely disseminate the virus throughout a susceptible population. We also found that parasitoid wasps participate to indirect transmission of densoviruses although probably in a non-specific manner. Altogether, these results are a first step toward the construction of a dynamic transmission model for densoviruses.

Contributed paper. Wednesday, 14:30 **186**

Discovery of circular single-stranded DNA viruses in top insect predators

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Viruses with circular single-stranded DNA (ssDNA) genomes that encode a replication initiator protein (Rep) are among the smallest viruses known to infect eukaryotic organisms. Additionally, their rapid evolution rates have led to the emergence of some of these viruses as serious pathogens. Recent research indicates that the host range of eukaryote-infecting circular Rep-encoding ssDNA (CRESS-DNA) viruses, which was previously thought to be restricted to plants and vertebrates, may include insects. To expand our knowledge of circular ssDNA viruses in invertebrates, this study surveyed CRESS-DNA viruses circulating among insect populations by targeting dragonflies (Epiprocta). Dragonflies are highly mobile top insect predators that accumulate viruses from their insect prey over space and time and, thus, can be used as 'sampling traps' to explore the diversity of CRESS-DNA viruses found among flying insects. Using degenerate PCR and rolling circle amplification coupled with restriction digestion, 16 CRESS-DNA viral genomes were recovered from eight different dragonfly species collected in tropical and temperate regions. Nine of the genomes are similar to cycloviruses and represent five species within this proposed genus, suggesting that cycloviruses are commonly associated with insects. Three of the CRESS-DNA viruses share conserved genomic features with the recently described fungal virus Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1. The remaining viruses are divergent species representing novel CRESS-DNA viral genera. The novelty of CRESS-DNA viruses identified in dragonflies using simple molecular techniques indicates that there is an unprecedented diversity of ssDNA viruses among insect populations.

Contributed paper. Wednesday, 14:45 **187-STU**

Single-stranded DNA viruses in marine crustaceans

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Metagenomic sequencing has recently revealed the ubiquity of eukaryotic circular single-stranded DNA (ssDNA) viruses in the marine environment; however, a definitive host has not been identified for most of these viruses. Through direct examination of marine shrimp and crab species, this study surveyed the diversity of circular ssDNA viruses in economically and ecologically important crustaceans, linking these newly discovered viruses to their hosts and improving our understanding of the ecological impact of ssDNA viral infection in marine crustaceans. Viral particles were partially purified from specimen homogenates through filtration, DNA was then

extracted and amplified through rolling circle amplification to enrich for small circular ssDNA templates. The concatenated circular genomes were then digested with restriction enzymes and the resulting products (~1– 4 kb) were cloned and sequenced. Thirteen distinct ssDNA viral genomes were recovered from five crab species and three shrimp species. Putative encoded proteins share less than 60% identity with known viral proteins from members of the *Circoviridae*. The detected genomes exhibit four different genomic architectures revealing an incredible diversity of ssDNA viruses in shrimp and crabs. Ongoing work aims to propagate these viruses in insect cell lines and develop a system to assess viral infectivity and modes of transmission.

Contributed paper. Wednesday, 15:00 **188**

Remarkable diversity of endogenous viruses in the genome of an isopod crustacean

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Recent studies in paleovirology have uncovered myriads of endogenous viral elements (EVEs) integrated in the genome of their eukaryotic hosts. These fragments result from endogenization, i.e., integration of the viral genome into the host germline genome followed by vertical inheritance. So far, most studies have used a virus-centred approach, whereby endogenous copies of a particular group of viruses were searched in all available sequenced genomes. Here we follow a host-centred approach whereby the genome of a given species (the crustacean isopod *Armadillidium vulgare*) is comprehensively screened for the presence of EVEs using all viral sequences available as queries. This search and downstream evolutionary analyses revealed that 56 EVEs corresponding to 11 different viral lineages belonging to 5 viral families (*Bunyaviridae*, *Circoviridae*, *Parvoviridae*, *Nimaviridae*, *Totiviridae*) and one viral order (*Mononegavirales*) became endogenized in *A. vulgare*. We show that viral endogenization occurred recurrently during the evolution of isopods, that *A. vulgare* viral lineages were involved in multiple host-switches that took place between widely divergent taxa. Furthermore, 32 *A. vulgare* EVEs have uninterrupted open reading frames, suggesting they result from recent endogenization of viruses likely to be currently infecting isopod populations. Overall, our work shows that isopods have been and are still infected by a large variety of viruses. It also extends the host range of several families of viruses and brings new insights into their evolution. More generally, our results underline the power of paleovirology in characterizing the viral diversity currently infecting eukaryotic taxa.

Contributed paper. Wednesday, 15:15 **189**

Iteraviruses (Densovirinae) from monarch and black swallowtail butterflies and slug caterpillar moths and characterization of their expression strategies

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Iteraviruses belong to a separate genus of the Densovirinae subfamily of the Parvoviridae family and includes three densoviruses, i.e. Casphalia extranea Densovirus (CeDNV), Dendrolimus punctatus Densovirus (DpDNV) and Bombyx mori Densovirus (BmDNV). In this study, we used a Sequence-Independent Single-Primer Amplification (SISPA) method to detect the pathogens of larvae from three additional insect species (Papilio polyxenes, Sibine fusca and Danaus plexippus), killed by some unknown pathogen. Sequencing of the clones that were obtained and BLAST analysis revealed the existence of three previously unknown densoviruses (provisionally named PpDNV, SfDNV and DppIDV). The genome of the new densoviruses were cloned into pCR2.1-topo or pBluescript(SK-) vectors. These virus sequences (including ITRs) have high identities with CeDNV and BmDNV. The identical genome organizations indicated that these three new densoviruses should be classified in the Iteravirus genus. Together with the infectious clones of CeDNV and BmDNV, we investigated the expression strategies of five different iteraviruses (PpDNV, SfDNV, CeDNV, BmDNV, DppIDV). Total RNA was obtained both from LD cell line transfected by infectious clones of the iteraviruses and virus infected larvae (Papilio polyxenes). RACE methods were used to identify the 5' and 3' transcription ends. The nonstructural (NS) and structural (VP) genes were located on the same strand of the genome. The NS cassette consists of two genes with NS1 and overlapping NS2. The NS2 transcripts all start at 7 nts downstream of the NS1 start codon. Transcription starts for NS1 genes are close to the AUG of NSS1. NS and VP transcripts do not overlap. The four VPs were similarly generated by leaky scanning translation of unspliced mRNA. The VP transcripts just start 2nts downstream of the poly (A) motif for NS transcripts. Interestingly, poly (A) signals for VP transcripts all overlap with the stop codons of the VP genes.

Contributed paper. Wednesday, 15:30 **190**

Remarkable genetic diversity of single-stranded DNA viruses in cultured shrimps and crickets

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Single-stranded DNA viruses are among the smallest viruses and include members of the *Parvoviridae* and *Circoviridae* families (linear and circular ssDNA viruses). In the past decades, PstDNV and AdDNV have been well-known viruses that have caused a severe impact on cultured shrimps and crickets. Here, we report the discovery and genome characterization of numerous novel denso- and denso-like viruses and, for the first time, new circoviruses from these hosts. During the last years, we received many cricket samples from North America that were negative for AdDNV. However, denso-like particles have been observed by EM. Complete sequence of different viral genome have been isolated and cloned including one circular ssDNA viruses of 2.5 kb, an ambisense densovirus of 4.9 kb and a segmented brevidenso-like virus (3.3 kb). Meantime, large numbers of new ssDNA viruses were also isolated from cultured shrimp from Vietnam. Characterization of these viruses revealed 3 different, unrelated circoviruses of 1.7, 1.7 (the latter is not using the standard genetic code and may have been ingested) and 1.3 kb. We also discovered a new shrimp parvovirus of about 4.1 kb that is phylogenetically poorly related to any known parvovirus. Near-atomic structures of some cricket and shrimp parvoviruses were obtained by X-ray crystallography as well as their transcription strategy. These results demonstrate a great diversity of ssDNA-viruses infecting these economically important animals. Future work will be focused on molecular features of theses viruses for a further insight into the evolution and classification of ssDNA viruses.

Contributed paper. Wednesday, 15:45 **191**

How do vine mealybug, grapevine leafroll-associated virus and grapevine interact on a molecular level?

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Vine mealybug (VMB), *Planococcus ficus*, is one of the most damaging grapevine pests in the world, largely because it is a vector of grapevine leafroll-associated virus (GLRaV). Since interactions among VMB, GLRaV and grapevine are responsible for the extent of VMB damage and GLRaV spread, we investigated the relationships among the three organisms. The effect of GLRaV infection on VMB was determined using cDNA-AFLP analysis and validated with RT-qPCR. It was found that VMB responds to GLRaV by activating only a few genes, and possibly also by endosymbiont mediation. The effect of VMB feeding on grapevine was investigated with microarray analysis and validated with RT-qPCR. Grapevine was found to respond to VMB feeding by mounting a weak response within a narrow window of time. These results are useful for understanding the interaction among the organisms in the VMB system, and limiting the damage caused in vineyards.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

BACTERIA 4

Contributed paper. Wednesday, 14:00 **192**

Analysis of the bacterial community of the insect pest *Lymantria dispar* during its life cycle

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Gypsy moth (*Lymantria dispar,* Lepidoptera) outbreaks can cause great damage to forestry across Europe. In order to control this pest, there is need for new insecticidal bacterial strains for the development of more effective biopesticides. The insect gut microbiota represents all aspects of microbial relationships, ranging from pathogenic to obligate mutualistic interactions. Latest investigations suggest that there is competition between individual opportunistic pathogens and that they are able to upregulate the production of virulence factors according to their density within hosts, what renders them interesting for use in combination with biocontrol agents.
The objective of this work is to characterize the bacterial midgut community of *L. dispar* and to monitor 1) changes in diversity during its life cycle, 2) changes within larvae from spring to summer and 3) differences between individuals. Microorganisms were first analyzed using a culture dependent approach where midguts were extracted and plated on media. Growing bacteria were analyzed by colony characteristics - color, size, shape, opacity, margin, elevation and viscosity and then by 16S rRNA gene sequencing. In a second step bacterial midgut communities were analyzed by a culture independent method using PacBio technology to sequence full length 16S rRNA genes.
Results showed relatively simple composition of the gypsy moth midgut community. We observed differences between individual larva from the same time point and structural changes of diversity in bacterial communities over the season.

This project is conducted within the frame of the SCIEX program with ETH Zürich and the University of Daugavpils as partners

Contributed paper. Wednesday, 14:15 **193**

Contacting microbe induce grooming behaviour in *Drosophila*

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Insects remove and clean microbes from their surface by grooming behavior, which is considered as a behavioral defense against pathogen/parasite infection in some cases. It is well known that the insects like *Drosophila melanogaster*, which live in an environment littered with bacteria, fungi and other microorganisms developing on decaying material devote a lot of time to self-grooming which seems to contribute cleaning their cuticula from external particles. The mechanisms that trigger this behavior are still ambiguous, although grooming behavior was identified in many insects. In this work, we examined if *D. melanogaster* can sense microbe in their habitat and if they conduct any hygiene behavior like grooming after they have perceived microbe. To follow the behavioral reaction, we focus on a contact chemo-stimulus, which would activate taste neurons. Microbe, microbe-related compounds and standard chemicals were used as stimuli and influence of water and mechanical stimuli were removed by using Gal4-UAS system in control experiments. Grooming seems to be specifically triggered by the activation of taste neurons since flies showed strong cleaning behavior when contacted with taste stimuli.

Contributed paper. Wednesday, 14:30 **194**

Cultivable gut bacteria of scarabs inhibit *B. thuringiensis* multiplication

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The entomopathogen *Bacillus thuringiensis* is used to control various pest species of scarab beetle but is not particularly effective. Gut bacteria have diverse ecological and evolutionary effects on their hosts, but whether gut bacteria can protect scarabs from *B. thuringiensis* infection remains poorly understood. To investigate this we isolated 32 cultivable gut bacteria from *Holotrichia oblita*, *Holotrichia parallela* and *Anomala corpulenta*, and analyzed their effect on *B. thuringiensis* multiplication and Cry toxin stability. 16S rDNA analysis indicated that these gut bacteria belong to the *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes* phyla. A confrontation culture analyses of the 32 isolates against three scarab specific *B. thuringiensis* strains showed that the majority of the scarab gut bacteria had antibacterial activity against the *B. thuringiensis* strains. The Cry toxin stability analysis results showed that whilst several strains produced proteases capable of processing the scarab-specific toxin Cry8Ea, none were able to completely degrade it. These results suggest that gut bacteria can potentially affect the susceptibility of scarabs to *B. thuringiensis* and that this should be considered when considering future control measures.

Contributed paper. Wednesday, 14:45 **195**

Interactions between the Med fly *Ceratitis capitata* (Wied.)and a new *Bacillus cereus sensu lato* strain

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The Med fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is a polyphagous species affecting many species of fruits and vegetables worldwide. Due to its high economical impact on crops, the management of this multivoltine pest is always necessary and mostly based on the application of various synthetic insecticidal formulations as foliage baiting or cover spraying. Besides the use of chemicals, the potential of entomopathogenic microorganisms (i.e. bacteria, fungi) against this pest has been highlighted. The lethal and sub-lethal effects of sporulated cultures of a novel *B. cereus sensu lato* strain lacking detectable *cry* genes and identified by its morphological and genetic features, have been studied in a larval based bioassay model. Sporulated cultures of this strain significantly reduced immature stages survival and development time, and the size of emerging Med fly adults. The toxicity has been associated to a specific parasporal fraction characterized through a proteomic approach (SDS-PAGE, 2D PAGE, LC MS/MS). The results of these analyses highlighted the possible role of different protein families produced also by other microbial entomopathogens and that have already been specifically associated to an insecticidal action. These proteins include molecular *chaperones* (GroEL), metalloproteases, aldehyde dehydrogenases, peptidases and other enzymes.

Contributed paper. Wednesday, 15:00 **196**

Long-term effect of *Bacillus thuringiensis* subsp. *israelensis* application on *B. cereus* group populations in Swedish riparian wetland soils

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The *Bacillus cereus* group (Bcg) commonly occurs in soil and includes the pathogens *B. cereus*, *B. thuringiensis* and *B. anthracis*, differing in pathogenicity and disease spectrum. The insect pathogenic *B. thuringiensis* subsp. *israelensis* (Bti) is available in products for augmentation biological control and has been applied worldwide to control larvae of the order Diptera. However, knowledge is limited on how long-term Bti application affects the structure of indigenous Bcg communities as well as the overall abundance of Bti. Based on new primer pairs targeting internal spacers located on the bacterial chromosome, group-specific quantitative PCR assays for Bcg and Bti in environmental samples were developed. On six occasions during the vegetation season, soil samples were collected in forest swamps and wet meadows which have been treated with Bti during the last 11 years as well as in untreated forest swamps, wet meadows and well-drained forests. Preliminary results from two of the time points indicate a decline of Bti abundance over time after the last treatment in wet meadows and forest swamps. These preliminary data also indicate that abundance of Bti in the untreated sites were lower than in the treated, independently of the sampling occasion. This study is coming up with the first specific PCR-primers for Bcg and Bti that target chromosomal DNA. These new tools will be useful for investigating the abundance and diversity of Bcg members in various environments and thereby for assessing the resident insecticidal potential of this bacterial group.

Contributed paper. Wednesday, 15:15 **197**

Proteomics of *Brevibacillus laterosporus* and its insecticidal action against noxious Diptera

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Brevibacillus laterosporus is a pathogen of invertebrates and an antimicrobial species, morphologically characterized by a typical spore surrounded by a firmly attached canoe-shaped parasporal body (CSPB). The biocontrol potential in agriculture of this bacterial species, is not limited to invertebrate pests (insects in different orders, nematodes and mollusks) but includes also phytopathogenic bacteria and fungi. This broad-spectrum activity is associated to a wide variety of molecules, including proteins and antibiotics, it produces. Whilst there are significant differences among strains in terms of virulence, the results of the recent whole genome sequencing of strains LMG 15441 and GI-9 revealed a conserved potential of this species to produce several polyketides, nonribosomal peptides, and toxins. Among genes encoding for putative toxins some show similarities to *Lysinibacillus sphaericus* mosquitocidal toxins.
Employing a *B. laterosporus-Musca domestica* bioassay model, associated to a proteomic and gene expression study, we have analyzed the implication in the microbial action of specific proteins produced during different bacterial life stages. Based on these results, new insights into the pathogenicity against noxious Diptera will be discussed.

Contributed paper. Wednesday, 15:30 **198-STU**

Outer membrane vesicles are vehicles for the delivery of *Vibrio* virulence factors to oyster immune cells

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V. tasmaniensis LGP32, a facultative intracellular pathogen of oyster hemocytes, was shown here to release outer membrane vesicles (OMVs) both in the extracellular milieu and inside hemocytes. Intracellular release of OMVs occurred inside phagosomes of intact hemocytes having phagocytosed few vibrios as well as in damaged hemocytes containing large vacuoles heavily loaded with LGP32. The OMV proteome of LGP32 was shown to be rich in hydrolases (29.8 %) including potential virulence factors such as proteases, lipases, phospholipases, hemolysins and nucleases. One major

caseinase / gelatinase named Vsp for vesicular serine protease, which is homologous to the VesA serine protease of *Vibrio cholerae*, was found to be specifically secreted through OMVs in which it is enclosed. Vsp was shown to participate in the virulence phenotype of LGP32 in oyster experimental infections. Finally, OMVs were highly protective against antimicrobial peptides, increasing the minimal inhibitory concentration of polymyxin B by 16-fold. Protection was conferred by OMV titration of polymyxin B but did not depend on the activity of Vsp or another OMV-associated protease. Altogether, our results show that OMVs contribute to the pathogenesis of LGP32, being able to deliver virulence factors to host immune cells and conferring protection against antimicrobial peptides.

Wednesday, 16:30-18:30

POSTERS

BACTERIA

Poster / Bacteria. Wednesday, 16:30. **BA-1**

A New Local Bio-Insecticide: Developing, Optimization, Toxicity and Determination of Activity

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The insects belonging to the order Coleoptera are one of the most harmful insect groups in our country and in all over the world. Members of coleopteran cause serious damages in the agricultural fields and the forested areas and the warehouses. So far, efforts to control coleopteran pests have mainly involved the use of chemical insecticides. These agents can have undesirable side-effects on humans, plant and other animal species, particularly predators and parasites of pests. In this study, we proposed to develop a biological preparation (bio-insecticide) against coleopteran pests using an insecticidal isolate of *Bacillus thuringiensis* subsp. *tenebrionis* (Mm2). Our results showed that the isolate has maximum growth at 30°C, at pH 7 in Tryptic Soy Broth containing 1% NaCl. Its sporulation was supported in synthetic medium and the bacterial cell suspension was produced in pilot fermenter. Powder bio-pesticide was produced using this cell suspension and necessary formulation materials in the spray dryer. The physical and biological properties like wettability, suspensibility, particle size, moisture content, and viable spores of the formulated powder were determined and noted as 24 s, 80%, 10 µm, 5% and $10x10^{12}$ (CFU/gdw), respectively. Insecticidal activity of the product against *Agelastica alni* and *Stophilus granarius* adults in laboratory conditions were investigated. Mortality results were identified as 37% against *S. granarius* and 100% against *Agelastica alni*.

Poster / Bacteria. Wednesday, 16:30. **BA-2**

`*Candidatus* Rickettsiella isopodorum´, a new lineage of intracellular bacteria infecting woodlice

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The taxonomic genus *Rickettsiella* (*Gammaproteobacteria; Legionellales*) comprises intracellular bacteria associated with a wide range of arthropods including insects, arachnids and crustaceans. Ultrastructural together with genetic evidence is provided for a *Rickettsiella* bacterium occurring in Germany in the common rough woodlouse, *Porcellio scaber* (Isopoda, Porcellionidae). The new bacterium is found very closely related to a *Rickettsiella* strain from California that infects the pill bug, *Armadillidium vulgare* (Isopoda, Armadillidiidae). Both bacterial isolates display the ultrastructural features described previously for crustacean-associated bacteria of the genus *Rickettsiella*, including the absence of well-defined associated protein crystals; occurrence of the latter is a typical characteristic of infection by this type of bacteria in insects, but has not been reported in crustaceans. As demonstrated by a molecular systematic approach combining multilocus sequence analysis (MLSA) with likelihood-based significance testing, both bacteria - despite their distant geographic origins - form a tight sub-clade within the genus *Rickettsiella*. In the 16S rRNA gene trees, this sub-clade includes other bacterial sequences from woodlice. Moreover, the bacterial specimens from *P. scaber* and *A. vulgare* are found genetically or morphologically different from each of the four currently recognized *Rickettsiella* species. Therefore, the designation `*Candidatus* Rickettsiella isopodorum´ has been introduced for this new lineage of isopod-associated *Rickettsiella* bacteria.

Reference: Kleespies R.G., Federici B.A., Leclerque A. Ultrastructural characterization and multilocus sequence analysis (MLSA) of `*Candidatus* Rickettsiella isopodorum´, a new lineage of intracellular bacteria infecting woodlice (Crustacea: Isopoda). Systematic and Applied Microbiology, in press.

Poster / Bacteria. Wednesday, 16:30. **BA-3-STU**

Analysis and characterization of binary AB toxins in the honey bee pathogen *Paenibacillus larvae*

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The gram-positive spore-forming bacterium *Paenibacillus larvae* is responsible for American foulbrood in honeybees. Four *P. larvae* genotypes could be distinguished via genotyping with ERIC-primers, ERIC I – IV, with genotypes ERIC I and II being frequently isolated form outbreaks worldwide. The most important phenotypic difference between the genotypes are the differences in virulence. Recent studies show that binary AB toxins play an important role in the infection mechanism, presumably in breaching the larval midgut epithelium as crucial step in pathogenesis. AB toxins usually consist of two subunits which are encoded either by the same or different open reading frames (ORF). The A subunit is enzymatically active and modifies a cellular target, e.g. by mono-adenosine diphosphate (ADP)-ribosylation. Contrarily, the B subunit is responsible for cell surface receptor binding and the translocation of the A subunit into the cell. Recently, two binary AB toxins, Plx1 and Plx2, have been identified as virulence factors in *P. larvae* ERIC I. The study on further binary AB toxins in *P. larvae* will be

continued via exposure bioassays with knockout mutants. We aim at analyzing and characterizing the binary AB toxins in *P. larvae* in order to gain further insight into the pathogenic mechanisms of *P. larvae.*

Poster / Bacteria. Wednesday, 16:30. **BA-4**

Interplay of Regulators Controlling Fit Insect Toxin Expression in the Biocontrol Bacterium *Pseudomonas protegens*

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The root-colonizing biocontrol agent *Pseudomonas protegens* CHA0 protect its plant hosts from fungal diseases by releasing toxic exoproducts into the rhizosphere. Remarkably, this microorganism might also function as a natural insecticide since it is capable of exhibiting potent oral and systemic insecticidal activity against various pest insect species. Recently, our group discovered an insecticidal protein toxin termed Fit, which makes essential contributions to insect killing. The Fit toxin gene *fitD* is located in a virulence cluster coding for a type I secretion system (FitA, FitB, FitC and FitE) essential for toxin transport and also for three regulators (FitF, FitG and FitH) of toxin expression. By using a Δ*fitF* Δ*fitG* Δ*fitH* triple mutant, in which each regulatory gene was individually reintroduced and expressed, we observed that the expression of the *fitABCDE* operon is positively regulated by the LysR-type regulator FitG and repressed by the response regulator FitH. We demonstrate that a phosphorylation of the conserved aspartate residue (D59) in the receiver domain of FitH is necessary to eliminate the repressive activity of the regulator, and that this residue is necessary for Fit toxin expression. Findings of an analysis of the heterologous expression of regulatory genes *fitG* and *fitH* in naturally Fit-locus deficient strains carrying a *gfp* reporter monitoring the *fitA* leader sequence activity strongly suggests that the LysR-type regulator FitG promotes Fit-toxin expression through specific binding to the promoter of the *fitABCDE* operon. These results allowed to improve the model explaining the regulation of Fit toxin expression in *P. protegens* CHA0.

Poster / Bacteria. Wednesday, 16:30. **BA-5-STU**

Identification and Characterization of *Bacillus thuringiensis* Strains with Nematicidal Activity

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Crystal proteins from the soil bacterium *Bacillus thuringiensis* (Bt) are globally used in agriculture as biological control agents against insect pest, but its use as a nematicidal control agent is still under development. In this work, a total of 310 Bt strains were screened for activity against the free-living nematode *Caenorhabditis elegans*. Strains LBIT-596 (serotype darmstadiensis) and LBIT-107 (serotype neoleonensis) showed significant toxicity levels. These strains were characterized by plasmid and RepPCR patterns, and flagellin gene sequencing. Preliminary bioassays of LBIT-596 and LBIT-107 spore-crystal complexes estimated LC_{50}s at 63.36 and 76.33 µg/ml, respectively, and 24.2 and 24.99 µg/ml, respectively, when pure crystals were tested. SDS-PAGE protein content analyses of LBIT-596 crystals showed two proteins (35 and 130 kDa) before activation, which turned into lower molecular-weight proteins (28 and 55 kDa) after activation. LBIT-107 also showed two major proteins of 28 and 70 kDa, before activation. Amplicons from the *cry*-gene conserved blocks and from *cyt1* gene group were cloned and sequenced. Sequence analyses indicated that LBIT-596 contains sequences identified within the *cry5B* and *cyt1A* gene families, while LBIT-107 contains sequences identified within the *cry14* and *cyt1A* gene families. Interestingly one of the amplicons from LBIT-107 showed only 88% identity with the *cry14A* gene. These results indicate a potential use of these toxins against economically important parasitic nematodes.

Poster /Bacteria. Wednesday, 16:30. **BA-6**

Evaluation of Culture media for maximal growth, Cry toxin production and insecticidal toxicity of *Bacillus thuringiensis*

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Bacillus thuringiensis Berliner is a gram positive soil dwelling, aerobic bacterium which produces parasporal crystal (Cry) toxins that are highly specific and effective against insect species. During the course of isolation of native strains, *B. thuringiensis* AUG-05 was found the most effective with a wide range of activity against lepidopterans. Hence, studies were carried out on its fermentation in different media to evaluate the production of maximal Cry toxin as well as spore and colony forming unit (cfu) counts. Increase in concentration of the Luria Bertani [(LB), composed of casein, yeast extract and sodium chloride in 2:1:2 w/w)] medium in the fermentation broth from 1 to 2% enhanced cfu, spore and also Cry1Ac and Cry2Ab toxin content. Addition of 1% Wesson salt in 1% LB broth dramatically increased spore, cfu counts, and also that of Cry1Ac but not of Cry2Ab. Spore and cfu counts in media were positively correlated with Cry1Ac and Cry2Ab contents. Bt powders from each fermentation with varying ratios of Cry1Ac and Cry2A toxins were more toxic to the cotton bollworm, *Helicoverpa armigera* than the tobacco caterpillar, *Spodoptera litura* and. Of all media substituting LB with agroproducts, most did well in supporting *B. thuringiensis* culture except for medium VI and VII, suggesting need for balancing qualitative and quantitative nutrients in the medium for optimal growth of the bacterium. Medium consisting of 2% wheat flour, 2% soybean meal and 1% Wesson salt could be considered as an alternative to LB medium to achieve economy of production costs.

Poster / Bacteria. Wednesday, 16:30. **BA-7**

Gene organization of large plasmids of novel mosquitocidal *Bacillus thuringiensis* TK-E6

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A novel *Bt* strain, TK-E6, isolated from grove soil in Japan, produces a mosquitocidal inclusion body called crystal consisting of several Cry proteins during sporulation phase. We detected twelve genes belong to the *cry* family, by degenerate PCR from *Bt*. TK-E6. Nucleotide sequences of these genes were determined and deduced ORFs encoding 140 - 145 kDa Cry proteins were cloned into a *Bt* expression vector carrying *cyt1A* promoter and *cry4A* terminator. Each Cry protein was purified and used for mosquitocidal assay against *Ae*. *aegypti* larva, any protein, however, did not show the strong activity when used alone. These results suggested that there was a synergistic action with some proteins for mosquitocidal activity. Pulse-field gel electrophoresis analysis showed that *Bt*. TK-E6 had five

plasmids ranging 66 - 224 mDa. Southern hybridization experiments revealed that twelve genes we detected had been distributed on four of five large plasmids. Interestingly, insertion sequences and transposon structures are also found in the up- and downstream of all genes. It is very possible that some DNA rearrangement of gene amplification occurred in both intra- and inter-plasmids during the evolutional process of *Bt*. TK-E6. Elucidation of structure of *Bt.* TK-E6 large plasmids is very important to know evolution of *Bt*. Therefore, we are analyzing the gene organization of large plasmids by the next generation sequencer.

Poster / Bacteria. Wednesday, 16:30. **BA-8-STU**

Testing of Vip3 proteins for the control of caterpillar pests

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Vip3 insecticidal proteins are produced by *Bacillus thuringiensis* during the vegetative growth phase and most of them have activity against lepidopteran species. Five *B. thuringiensis* Vip3A proteins (Vip3Aa, Vip3Ab, Vip3Ad, Vip3Ae and Vip3Af) and their corresponding trypsin-activated toxins were tested for their toxicity against eight lepidopteran pests: *Agrotis ipsilon*, *Helicoverpa armigera*, *Mamestra brassicae*, *Spodoptera exigua*, *Spodoptera frugiperda*, *Spodoptera littoralis*, *Ostrinia nubilalis* and *Lobesia botrana.* Vip3Aa, Vip3Ae and Vip3Af were the most active proteins. Vip3Af was the protein active against most of the species tested. Contrarily, Vip3Ad was non-toxic to any species. *Agrotis ipsilon* was the species most susceptible to the four active proteins, whereas *O. nubilalis* was tolerant to all Vip3 proteins tested, with just some susceptibility to Vip3Af. The results obtained will help to design new combinations of insecticidal protein genes in transgenic crops or in recombinant bacteria for the control of insect pests.

Poster / Bacteria. Wednesday, 16:30. **BA-9**

Interactions between Cry and Vip proteins from *Bacillus thuringiensis* against different lepidopteran pests

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Second generation *Bt* crops (insect resistant crops carrying *Bacillus thuringiensis* genes) combine more than one gene coding for insecticidal proteins in the same plant to provide a better control of agricultural pests. Some of the new combinations involve co-expression of *cry* and *vip* genes. Since Cry and Vip proteins have different midgut targets and possibly different mechanisms of toxicity, it is important to evaluate possible synergistic or antagonistic interactions between these two classes of toxins. Three members of the Cry1 class and three from the Vip3A class were tested against *Heliothis virescens* for possible interactions. At the level of LC_{50}, Cry1Ac was the most active protein, whereas the rest of proteins were similarly active. However, at the level of LC_{90}, Cry1Aa and Cry1Ca were the least active proteins, and Cry1Ac and Vip3A proteins were not significantly different. In the experimental conditions used, we found an antagonistic effect of Cry1Ca with the three Vip3A proteins and a slight antagonism of Vip3Af with either Cry1Aa or Cry1Ac. The interaction between Cry1Ca and Vip3Aa was also tested on two other lepidopterans. Whereas antagonism was observed in *Spodoptera frugiperda*, synergism was found in *Diatraea saccharalis.* In all cases, the interaction between Vip3A and Cry1 proteins was more evident at the LC_{90} than at the LC_{50} level. The fact that the same combination of proteins may result in a synergistic or an antagonistic interaction may be an indication of different types of interaction with the host depending on the insect species tested.

Poster / Bacteria. Wednesday, 16:30. **BA-10**

Cry1Ac and Cry1F toxicity and binding sites study in two important soybean pests, *Anticarsia gemmatalis* and *Chrysodeixis (=Pseudoplusia) includens.*

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Anticarsia gemmatalis (velvetbean caterpillar) and *Chrysodeixis (=Pseudoplusia) includens* (soybean looper) are two important defoliating insects of soybean that cause economic damage in soybean producing regions in the Americas. Both lepidopteran pests are currently controlled mainly with synthetic insecticides. Alternative control strategies such as biopesticides based on the *Bacillus thuringiensis* (Bt) toxins or transgenic plants expressing Bt toxins can be used and are increasingly being adopted. The studies on the insect susceptibility and mode of action of the different Bt toxins are crucial to determine management strategies to delay insect resistance. Also, these studies are necessary to help design pyramided transgenic plants involving more than one Bt toxin to ensure a crop long term protection.
In the present study the susceptibility of both soybean pests to Cry1Ac and Cry1F has been investigated. Bioassays performed in larvae show that both insects are susceptible to these two toxins. Competition-binding studies using brush border membrane vesicles indicate that Cry1F and Cry1Ac share some, but not all, binding sites in midguts of both insects. Incomplete shared binding indicates that there are resistance management benefits from combining the two proteins in Bt soybeans. Additional information on the receptors involved in binding and consequent cross-resistance potential are needed to more fully understand the long-term durability of combinations of Cry1Ac and Cry1F to control these two pests.

Poster / Bacteria. Wednesday, 16:30. **BA-11-STU**

***In vivo* and *in vitro* binding of Vip3Aa to *Spodoptera frugiperda* midgut and characterization of binding sites using ^{125}I-radiolabeling**

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Bacillus thuringiensis vegetative insecticidal proteins (Vip3A) have been recently introduced in important crops as a strategy to delay the emerging resistance to the existing Cry toxins. The mode of action of Vip3A proteins has been studied in *Spodoptera frugiperda* with the aim to characterize their binding to the insect midgut. Histological localization of Vip3Aa in the

midgut of intoxicated larvae using immunofluorescence showed that Vip3Aa bound to the brush border membrane along the entire apical surface. The presence of fluorescence in the cytoplasm of epithelial cells seems to suggest internalization of Vip3Aa or a fragment of it. Successful radiolabeling and optimization of the binding protocol for the ^{125}I-Vip3Aa to *S. frugiperda* BBMV allowed the determination of binding parameters of Vip3A proteins for the first time. Heterologous competition was performed using different protein competitors with the aim to determine if they share the same binding sites with Vip3Aa in *S. frugiperda* BBMV and thus select the appropriate candidates to be used in combination with the later in transgenic crops.

Poster / Bacteria. Wednesday, 16:30. **BA-12**

Comparative histopathology of two novel bacterial insecticidal proteins in *Tenebrio molitor* and *Diabrotica virgifera virgifera* larvae

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Larvae of the Western corn rootworm (*Diabrotica virgifera virgifera*) are the most devastating pest of corn in the US. Due to reports of field-evolved resistance, novel insecticidal proteins are needed as alternative candidates for expression in transgenic corn to control this insect pest. A novel insecticidal protein from a Gram negative bacterium (toxA) and a Cry-derived protein (toxB) have been identified and developed, respectively, as candidates for expression in transgenic corn targeting larvae of *D. v. virgifera*. In this work, we used *Tenebrio molitor* larval midgut as a model to characterize toxin binding and histopathology of toxA and toxB proteins in coleopteran larvae, and then compared to histopathology in *D. v.virgifera* larval midguts. While both toxins bound to the midgut brush border membrane, differences observed in H&E stained histological sections and TUNEL assays support differences in the mode of action of these toxins in coleopteran larvae.

Poster / Bacteria. Wednesday, 16:30. **BA-13-STU**

Role of ABC-C2 in the interactions of *Heliothis virescens* with its host plants and Bt toxins

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Bacillus thuringiensis (Bt) Cry toxins are widely used biopesticides for reduction of crop losses caused by larvae from species such as *Heliothis virescens*. Until recently, Cadherin was identified as the major receptor for Bt toxins, albeit Bt resistance was shown to be genetically linked to an inactivating mutation in an ABC transporter. ABC (ATP-binding cassette) transporters are transmembrane proteins that hydrolyze ATP in order to conduct transport and other cellular processes. To date, we have no insights into the physiological role of this specific ABC transporter as well as into its role in the Bt toxin mode of action. We aim to investigate whether ABC-C2, the specific ABC transporter implicated in Bt resistance, acts as a receptor to Cry toxins. Furthermore, we want to find out whether an inactivated (mutated) ABC-C2 could cause a trade-off between Cry toxins and host plant secondary metabolites in Bt resistant insects. To address these two hypotheses, we first heterologously express *H. virescens* Cadherin and ABC-C2 in Sf9 cells. In addition, feeding assays with two *H. virescens* populations, JEN2 (wild type) and YEE (ABC-C2 mutant), are performed with different host plants as well as host plant secondary metabolites incorporated into artificial diet. The genes of interest were expressed successfully, generating the basis for our ongoing *in vitro* trials. Subsequently, the effect of different Cry toxins on transfected cells will be investigated. Our first feeding assays with homozygous Bt susceptible and homozygous Bt resistant insects revealed a trade-off between Cry toxins and host plant secondary metabolites

Poster / Bacteria. Wednesday, 16:30. **BA-14-STU**

AminomemtidaseN in *Popillia japonica* Newman larvae is putative *Bacillus thuringiensis* Cry8Da toxin receptor

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Cry8Da from *Bacillus thuringiensis galleriae* SDS-502 has insecticidal activity against both the larvae and adult of Japanese beetle (*Popillia japonica* Newman). The receptor determines the specificity of the insecticidal activity of Cry proteins and hence, in order to reveal the mode of action of Cry toxin, receptor identification is a necessary step. However, a receptor for Cry8-type toxin has not been identified in the Scarabaeidae family of insects. Therefore, we aimed to identify the receptor of Cry8Da toxin in larvae *P. japonica* BBMV. A ligand blot showed the Cry8Da toxin bound to 110 kDa and 40 kDa protein in the BBMV of larvae *P. japonica*. The 110 kDa protein had higher binding affinity than the 40 kDa protein. In order to identify the Cry8Da toxin binding protein in the BBMV of larvae *P. japonica*, it was purified by column chromatography. The result of mass spectrometry indicated that the Cry8Da toxin binding protein in the BBMV of larvae was aminopeptidaseN which is commonly reported as receptors for Cry toxins in Lepidopteran and Dipteran insects. The 106 kDa APN homologous genes in larvae *P. japonica* could be amplified by PCR using degenerate oligonucleotide primers designed from a conserved sequence of Coleopteran APN. The 106 kDa APN is truncated into two peptides and tested to confirm the ability of binding with Cry8Da toxin. This experiment indicated the APN in larvae *P. japonica* is the receptor for Cry8Da toxin.

Poster / Bacteria. Wednesday, 16:30. **BA-15**

A Whole Genome Approach to Determine Cadherins associated with Bt toxicity in the Diamondback Moth, *Plutella xylostella*

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Diamondback moth, *Plutella xylostella*, is a main pest of Brassicaceae throughout worldwide and was first reported to evolve resistance to Bt toxins in field population. Cadherin has been known to be one of receptors of *Bacillus thuringiensis* Cry proteins and synergizes Cry toxicity against Lepidopteran, Dipteran, and Coleopteran insects by elevating a toxin oligomerization. Full genome analyses of several model insects suggest various number of cadherin genes in an organism and raise a fundamental question on which cadherin(s) is the Bt receptor. In a whole genome sequence of *P. xylostella*, 52 open reading frames were annotated to be cadherins, in which putative Bt receptors were chosen on the basis of three receptor motifs: a signal peptide, cadherin repeat, and transmembrane domains. Compared to other cadherins of *P. xylostella*

(PxCads), *PxCad1* has the highest homology with other lepidopteran insect cadherins previously associated to the Bt mode of action. *PxCad1* was expressed in all developmental stages especially in gut tissue. Expression of *PxCad1* was suppressed by feeding its specific double-stranded RNA (dsPxCad1) in the third instar. The suppression of *PxCad1* expression did not significantly influence on pupal and adult developments of *P. xylostella*. However, the larvae treated with dsPxCad1 (150 ng/larva) significantly reduced susceptibility to *B. thuringiensis* Cry1Ac toxin. In contrast, the dsPxCad1-treated larvae did not show any change in susceptibility to *B. thuringiensis* Cry1Ca toxin. Only one cadherin, PxCad1, out of 52 candidate cadherins is the Bt receptor and is responsible for the specificity to Bt toxin, Cry1Ac.

Poster / Bacteria. Wednesday, 16:30. **BA-16**

RNA Interference of Integrin subunit β1 Impairs Development and Immune Responses of the Oriental tobacco budworm, *Helicoverpa assulta* against Bacteria

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Integrin is a cell surface protein that is composed of α and β heterodimer and mediates cell interaction with extracellular matrix or other cells including microbial pathogens. A full length cDNA sequence (2,517 bp) of a integrin subunit β1 (*HaITGβ1*) was cloned from the oriental tobacco budworm, *Helicoverpa assulta*. Phylogenetic analysis showed that *HaITGβ1* was clustered with other insect β integrin subunits with the highest amino acid sequence identity (61%) to β1 of other Noctuidae such as *Spodoptera exigua* and *S. litura*. Structural analysis of the *HaITGβ1* possessed all functional domains known in other insect β1 integrins. RT-PCR analysis showed that *HaITGβ1* was expressed in all developmental stages and all tested tissues of *H. assulta*. Injection of double-stranded *HaITGβ1* RNA (dsHaITGβ1) into third instar of *H. assulta* suppressed *HaITGβ1* expression and resulted in significant delay from last larval stage to pupal stage. The dsHaITGβ1 injection significantly impaired nodule formation of *H. assulta* in response to bacterial challenge and hemocyte adherence. These results suggest that *HaITGβ1* plays crucial roles in cellular immune responses as well as development in *H. assulta*.

Poster / Bacteria. Wednesday, 16:30. **BA-17**

A natural hybrid of a *B. thuringiensis* Cry2A toxin implicates domain I in specificity determination.

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A PCR-RFLP method was used to identify *cry2A* toxin genes in a collection of 300 strains of *Bacillus thuringiensis* were confirmed with *cry2* gene. Of the 81 genes identified the vast majority appeared to be *cry2Aa* (32) and *cry2Ab* (46) on the basis of their RFLP pattern. Three genes showed a different pattern and were subsequently cloned and sequenced. The gene cloned from strain HD395 was named *cry2Ba2*. The proteins encoded by the genes cloned from LS5115-3 and DS415 shared enough similarity with existing toxins that their genes were named *cry2Aa17* and *cry2Ab29* respectively by the toxin nomenclature committee. Despite this overall similarity these two toxins resembled natural hybrids with Cry2Ab29 resembling Cry2Ab for the majority of the protein but then showing identity to Cry2Aa for the last 60 amino acids. For Cry2Aa17, domains II and III resembled Cry2Aa whilst domain I resembled Cry2Ab. The toxicity of the recombinant toxins against three insects was tested, and it was found that the toxicity of Cry2Aa17 more closely matched the toxicity profile of Cry2Ab than that of Cry2Aa, thus implicating domain I in specificity determination. Analysis of all publically available Cry2Aa sequences identified other examples of natural hybrids.

Poster / Bacteria. Wednesday, 16:30. **BA-18**

***Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of *Crioceris quatuordecimpunctata* to *Beauveria bassiana* infection**

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The spotted asparagus beetle, *Crioceris quatuordecimpunctata* (Coleoptera: Chrysomelidae), is one of the most devastating pests of asparagus in China. Sprayed synthetic pesticides have been used to control *C. quatuordecimpunctata* damage, but they pose problems because of residues and harm to natural enemies. Neither the microbial coleopteran-specific toxin from *Bacillus thuringiensis tenebrionis*, Cry3Aa, nor the fungal pathogen Beauveria bassiana have sufficient activity to effectively control *C. quatuordecimpunctata* damage to asparagus. However, second instar C. quatuordecimpunctata larvae exposed to a sublethal dose of Cry3Aa toxin demonstrated significantly higher larval mortality when exposed to B. bassiana. Our results suggest that a combination of Cry3Aa and *B. bassiana* may be effective in reducing damage by *C. quatuordecimpunctata* larvae to asparagus.

Poster / Bacteria. Wednesday, 16:30. **BA-19**

InterVening Sequence (IVS) elements as genetic markers for the differential diagnosis of arthropod-associated *Rickettsiella* bacteria

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Genomic analysis has revealed the presence of insertion sequences within 23S ribosomal RNA encoding genes of arthropod-associated *Rickettsiella* bacteria (*Gammaproteobacteria*). Secondary structure modelling shows that these insertions fulfill the structural criteria for RNase III processed bacterial intervening sequence (IVS) elements.
IVS elements have previously been identified within the rRNA operons of several *Alphaproteobacteria* and occur comparatively frequently within *Enterobacteriaceae*, but not in *Escherichia coli*. In these bacteria, IVS insertion sites have been shown to be conserved with respect to deduced rRNA secondary structures. 23S rRNA gene insertions in *Rickettsiella* occur at one of these conserved loci, more exactly within rRNA helix 25, and at a previously unidentified insertion site within helix 72.
Expression of the *Rickettsiella* 23S rRNA genes in the surrogate host *E. coli* by a plasmid replacement approach leads to rRNA fragmentation and thereby confirms that *Rickettsiella* insertion sequences at both sites can function as IVS elements. Given the

lack of sequence similarity with current GenBank database entries, IVS25 and IVS72 give rise to two unprecedented IVS element superfamilies. Whereas the IVS72 element is highly conserved across the full range of investigated *Rickettsiella* species and *Rickettsiella*-like bacteria, the sequence of element IVS25 strongly varies among different *Rickettsiella* strains. Using the sequence information available for both IVS elements, a PCR-based approach for the genus-specific identification and infra-generic characterization of *Rickettsiella* bacteria has been developed.

Poster / Bacteria. Wednesday, 16:30. **BA-20**

Type IV Secretion System (T4SS) substrates as potential virulence factors of arthropod-pathogenic *Rickettsiella* bacteria

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Rickettsiella bacteria (*Gammaproteobacteria: Legionellales*) are intracellular pathogens of arthropods that multiply inside replicative vacuoles within host cells. Delivery of bacterial proteins across the vacuole membrane to the host cell's cytosol is believed to be of key importance for successful infection and pathogenesis.
Comparative genomic analysis of *Rickettsiella* and related bacteria has revealed the presence of a complete set of gene clusters presumably encoding a type IVB secretion system (T4SS) in two *Rickettsiella* strains of the pathotypes `*R. melolonthae´* and `*R. armadillidii´*, i.e. infecting, respectively, the European cockchafer and the pill bug. Hypothetical *Rickettsiella* T4SS key components show high similarity to orthologs in the Dot/Icm systems of the related vertebrate pathogens *Legionella pneumophila* and *Coxiella burnetii*, and T4SS gene cluster organization is very similar in these bacteria. In *Legionella* and *Coxiella*, involvement of Dot/Icm systems and several of their substrates into infection and pathogenesis has been demonstrated previously. In *Legionella*, transcriptional regulation of both T4SS structural and substrate genes is most likely mediated by several bacterial two-component systems, but only one of these, PmrAB, seems to be conserved in the genomes of both *Coxiella* and *Rickettsiella*. Expression studies in the surrogate host *Escherichia coli* that lacks an own T4SS, have demonstrated that `*R. melolonthae´* PmrAB drives expression from the promoter regions of the presumed homologous T4SS gene clusters. Comparative *in silico* analysis of PmrAB regulons reveals a very high degree of divergence in hypothetical T4SS substrates sets that is in line with expectations from the specific host-adaptation of these bacterial pathogens

Poster / Bacteria. Wednesday, 16:30. **BA-21**

Unbalanced Polyphosphate Levels Impair Insect Pathogenicity in Plant-Beneficial *Pseudomonas protegens*

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Pseudomonas protegens is a plant-associated bacterium with lifestyles that potentially may be exploited for its use as a biological control agent in agricultural applications. The bacterium is a highly competitive root colonizer and produces antifungal compounds that ward off soil-borne plant pathogenic fungi and oomycetes. *P. protegens* is also capable of killing larvae of various pest insects following oral or systemic infection. We are exploring global regulatory mechanisms that control insect pathogenicity of the plant-beneficial bacterium. Here, we provide evidence that altering cellular levels of polyphosphate (PolyP) may strongly impair insect pathogenicity in *P. protegens.* The polymer is known for its involvement in regulation of diverse cellular and metabolic processes contributing to bacterial survival and virulence. *P. protegens* mutants with deletions in *ppk1*, encoding a PolyP kinase, or *ppx*, encoding an exopolyphosphatase, had a markedly reduced capacity to kill larvae of the Large White *Pieris brassicae* following oral infection. Oral toxicity could be restored by reintroducing the respective intact alleles into the mutant strains. Deletion of *ppk1* or *ppx* resulted in reduced in situ expression of a major virulence factor required for insect pathogenicity in *P. protegens*, i.e. the insecticidal toxin Fit, in insect larvae. We hypothesize that altering PolyP levels affects stress tolerance of *P. protegens* in the insect host thereby impacting virulence of the bacterium.

Poster / Bacteria. Wednesday, 16:30. **BA-22-STU**

***Paenibacillus larvae* and the virulence factor SplA- an ERIC II specific S-layer Protein**

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Paenibacillus larvae is the causative agent of the notifiable epizootic American Foulbrood of honey bees. Four genotypes, ERIC I - IV of this pathogen do exist, with only ERIC I and II being frequently isolated from outbreaks worldwide. Despite the importance of the disease, molecular and cellular details of pathogen-host interaction during pathogenesis of AFB in honey bee larvae are poorly understood. Recently, the surface layer protein SplA was identified and functionally characterized as the first virulence factor of the *P. larvae* genotype ERIC II. Through a gene-disruption strategy expression of the *spl*A-gene was successfully interrupted. In infection assays, SplA-deficient *P. larvae* and the parental wild-type bacteria were compared and it was demonstated that lack of SplA expression resulted in a significant decrease in total mortality. To futher investigate the role of SplA in virulence of P. larvae, SplA has been expressed in in the natural SplA-deficient genotype *P. larvae* ERIC I. We will present our most recent data on this SplA-expressing ERIC I-mutant in respect to growth characteristic in the lab and in larvae and, most importantly, to virulence parameters in exposure bioassays when compared to the parental wild type strain and to naturally SplA-expressing ERIC II.

Poster / Bacteria. Wednesday, 16:30. **BA-23**

Influence of (varying) population size on host-parasite coevolution: an experimental approach

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Host-parasite interaction is one of the most common and important type of interaction among species, which has a strong impact on species evolution. The signatures of this impact have been identified in genomes, natural communities and on a phylogenetic level. It is not surprising that many aspects of host-parasite relationships have received particular attention from evolutionary biologists. Paradoxically, one indispensible and basic property of host-parasite interaction, population size oscillations, has been overlooked as a factor in host-parasite

coevolution. Parasites, by reducing host fecundity and survival, strongly affect population size of the host, which very often is their only ecological niche. Already in the 1920s Lotka and Volterra showed that antagonistic interactions between species would lead to interdependent oscillations in their population size. However, most of the current models of host-parasite coevolution ignore population size changes or use a deterministic approach which cannot realistically imitate the finite nature of real populations. Similarly, in most experimental studies on host-parasite coevolution the population size is kept constant as a matter of good practice. To enhance a more realistic understanding of the coevolutionary dynamics, we performed laboratory-controlled evolution experiments with the model nematode host *Caenorhabditis elegans* and its microparasite *Bacillus thuringiensis* and specifically varied the factor population size. Here, we will show our results on temporal changes in host fitness and parasite virulence under different population size regimes.

Poster / Bacteria. Wednesday, 16:30. **BA-24**

An *in vivo* experimental evolution system for analyzing bacterial adaptation and evolution of *Bacillus cereus sensu lato* in an insect model

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The continuous exposition of a pathogenic bacterium in a host during a serial passage experiment (SPE) may drive the fixation of mutations that favour its growth and multiplication in the host environment [1]. These changes that are usually associated with an increase in virulence, can be now traced during an SPE by whole genome sequencing of the evolved variants [2]. Here we describe the set up and initial results of a SPE using a *Bacillus thuringiensis* crystal minus strain (Bt407 Cry-) [3] using *Galleria mellonella* larvae. A new infection protocol has been established which permits bacterial multiplication inside the intestine following force-feeding with spores. The genomes of experimentally evolved bacteria that show significant changes in virulence or persistence will be sequenced and compared with the initial parental strain. Such a global genome based approach of pathogen evolution analysis should allow us to describe the history of the events which arose during the evolution of the *B. cereus* group in one of its natural hosts and explain phenotypic variations based on genotypic differences.

DISEASES OF BENEFICIAL INVERTEBRATES

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-1-STU**

Identification and Characterization of Immune Inhibitor A Metalloprotease of the Honey Bee Pathogen *Paenibacillus larvae*

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Honey bees (*Apis mellifera*) are essential pollinators of various agricultural crops and fruit but also of many wild plants. Therefore, it is crucial to maintain honey bee health and prevent or cure diseases. The most contagious and fatal bacterial disease of honey bee brood is American Foulbrood (AFB) caused by *Paenibacillus larvae*, a Gram positive, spore-forming bacterium. Infection spreads among the whole hive, eventually leading to the loss of entire colonies resulting in considerable losses in apiculture. Despite the enormous impact of this disease and intensive research, molecular mechanisms involved in the pathogenesis are still not fully understood. Recently we have identified and characterized four genotypes of *P. larvae* (ERIC I-IV) which differ, among other factors, in virulence. Here we present our data on immune inhibitor A (InhA), a metalloprotease which is exclusively secreted by *P. larvae* ERIC II. In homologs of other pathogenic bacteria, InhA has been shown to have multiple functions such as degradation of anti-microbial peptides and cleavage of tight junctions. Here we functionally characterize InhA of *P. larvae* by combining transcriptomic, proteomic and histological studies as well as *in vivo* exposure bioassays with wild type and mutant P. larvae, the latter being deficient in InhA expression.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-2**

Awareness and Concept of Insects in a Korean Population

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To investigate the degree of individuals' concept and awareness of insects, a survey study was conducted with students and adults living in Korea. The misconception rate for insects was about 50% for both students and adults, but it was lower for students and people who had experienced insect-related events than for adults and those who had not. The highest misconception rate was obtained in answer to a question about the basic structure of an insect. Most people had a high preference of insects. Significant differences and correlations for the preference of insects were found between students and adults, men and women, people who had experienced insect-related events and those who had not. The experience of an insect-related event most influenced preference of insects. These results suggest that increasing people's interest in insects and utilizing insects in treatment situations may be beneficial for the field of mental healthcare.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-3**

Virus Epizootiology in Managed and Native Bee Populations

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The possible cross host-genus transmission of several honeybee viruses into native bee populations has recently been proposed. Given current pollination practices and the number as well as high levels of different viruses found in honeybees, the cross genus transmission of these viruses could have a dramatic impact on the health of native bees. In order to examine this possibility we initiated a study of the prevalence of the two honeybee viruses; deformed wing virus (DWV) and black queen cell virus (BQCV) in *Apis* and *Bombus sp.* where *Apis* was maintained under different conditions. These included sites

where stationary or migratory *Apis* hives were present, and sites where no or few *Apis* were present. Both viruses were found in both bee species in sites were *Apis* hives were present. The level of BQCV was significantly higher than DWV in all sites in both bee species when present. While BQCV reached level of 100% in *Apis* and 80% in *Bombus* in both migratory and stationary sites, DWV levels were only at 60% in *Apis* and 30% in *Bombus* in these sites. In the no or few *Apis* sites, BQCV reached levels of up to 65% in *Bombus* and DWV was never found in more than 10% of these bees. We are currently examining gene sequences of viruses recovered from the different bee species collected at each site to determine if they cluster by bee species or by collection site thereby providing further evidence on the interspecies transmission of these two viral pathogens.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-4**

Honeybee Virus Epizootiology in Bee Populations in Connecticut, USA

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We examined the prevalence of Black Queen Cell Virus (BQCV), and Deformed Wing Virus (DWV) in bee pollinators found foraging on pumpkins (*Cucurbita sp*.) on four farms in Connecticut. The three main groups foraging on pumpkins, *Apis mellifera*, *Bombus sp*. and *Peponapis pruinosa,* were sampled 5 times at each site from early June to late September. Sampling included approximately 20 bees of each group when available. Our initial analysis has focused on BQCV which is the most prevalent of the viruses in bees we have examined to date. Of the ~ 1,000 bees we have analyzed to date, 46.3% were found to be infected with BQCV. This virus was the most prevalent in *Apis* with 73.2% being infected, while the *Bombus* and *Peponapis* were infected at 37.7% and 3% respectively. The level of virus-positive bees of any species from the different farms ranged between 2.3% and 91.9% and overall our results suggest a correlation between the level of this virus in honey bees and the level of infection of other bee species. On the two farm sites where we found honey bees infected with BQCV at 95% and 75%, *Bombus* bees were at 91% and 40% respectively. At the site where we found only 10% of *Apis* infected with BQCV we were able to detect only 9.5% BQCV infected *Bombus*, suggesting that the infection of *Apis* and *Bombus* is clustered and may be connected in some way.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-5**

High-throughput sequence analysis of the change in expression profile of Ig2-, Ig3- and Ig7- variant domains in *Carcinus maenas* Down Syndrome Cell Adhesion (*CmDscam*) mRNAs in response to pathogenic infection

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Previously, we have identified a DSCAM gene (*CmDscam*) within the shore crab *Carcinus maenas*. This gene codes for a pattern recognition protein and has alternately spliced Ig2-, Ig3- and Ig7- domains and multiple different 3' UTRs. In other decapods evidence that these variable domains are alternately spliced during an immune response has been used to support a concept of specificity within arthropods. However, these data have been generated using conventional Sanger sequencing of a limited number of clones. This approach has insufficient depth to confirm unequivocally that the transcript profile is changed specifically through infection. Herein we present the first high throughput sequencing comparison of the variant Ig2-, Ig3- and Ig7- domains in response to Gram-negative or Gram-positive bacterial challenge. Haemolymph from individual crabs was sampled before and after a single sub-lethal inoculation with either bacterium to produce a deep haemocytopenia through haemocyte degranulation. Amplicons from each sample were then deep sequenced to test the hypothesis that bacterial infection specifically alters the transcription of *CmDscam* during the immune response. Data are discussed in light of new theories of specificity and memory within the innate immune system of decapods.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-6** ,

A novel pathogenic *Paenibacillus* strain of *Biomphalaria glabrata,* an intermediate host for schistosomiasis

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Schistosomiasis is the second most widespread tropical parasitic disease after malaria. To achieve the objective of schistosomiasis eradication in a decade, various research strategies and treatment programs were recommended and supported by WHO. One of these applicable approaches is based on the control of snail vectors in endemic area. Previous field studies have shown that competitor or predator introduction could be effective but no systemic investigation has ever been conducted to identify snail microbial pathogen and evaluate its molluscicide effect. In our laboratory, infectious agent was isolated on white nodules from unhealthy Biomphalaria snails. Only one bacteria was characterized and identified as Paenibacillus sp closely related to *P. alvei* through 16S and rpoB DNA analysis. Histopathological examination has shown massive bacterial infiltration leading to an overall disorganization of snail tissues. Exposure of healthy snails to Paenibacillus infected snails led to a massive mortality. Moreover, the number of hatched snails was significant lower in exposed snails than in control whereas the spawning appeared to be unaffected. Embryonic lethality is correlated with the presence of this pathogenic bacteria in eggs. This study reports the first description of a novel paenibacillus strain as snail microbial pathogen by affecting both adult and embryonic stages.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-7**

Venom from the ectoparasitic wasp *Habrobracon hebetor* activates calcium-dependent processes of haemocytic degradation in *Galleria mellonella* larvae

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The influents of *Habrobracon hebetor* venom on the cellular and humoral immune reactions of the wax moth larvae (*Galleria*

mellonella) by the naturally envenomation were analyzed. A strong decrease of phenoloxidase (PO) activity in the haemolymph and the number of haemocytes with PO activity of envenomated larvae were recorded. The capsule melanization in the envenomated larvae was twofold less than in control. Production of reactive oxygen species in the haemolymph of envenomated larvae also decreased. The main immune reactions (capsule formation, phagocytosis and coagulation of the lymph) are directly related by emission of calcium ions (Ca^{2+}) into the cytosol and in the pericellular space of haemocytes. The cytosolic calcium concentration in the haemocytes of *G. mellonella* larvae on first and second day after envenomation from *H. hebetor* female was measured (fura - 2 AM used). The increase of Ca^{2+} concentration and phospholipase C activity in haemocites were registered for two days after envenomation. The addition of the parasitic venom *in vitro* (final concentration of protein 6,2 µg/ml) have induced the decreasing of viability and adhesive capacity of the haemocytes during one hour. The membrane potential was measured with a fluorescent probe. The changes of trans-membrane potential of hemocytes were investigated both *in vitro* and *in vivo* experiments. The degree of trans-membrane potential was in direct dependence of the added venom concentration. The envenomated insects exhibited the decreased potential values.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-8**

Histopathological analyses of different tissues of diseased honey bees (*Apis mellifera*)

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The western honey bee (*Apis mellifera*) is threatened by numerous infectious pathogens (bacteria, viruses and fungi), affecting different life stages of the honey bee and various tissues. This work aims to compare diseased and healthy honey bee tissues in order to detect the pathogens' specific localization and to identify tissue alterations caused by various etiologic agents. For this purpose honey bee larvae were infected with *Paenibacillus larvae*, the causative agent of American Foulbrood (AFB), a notifiable epizootic, by feeding first instar larvae with *P. larvae* spores of different genotypes. Also, white eyed pupae were infected with deformed wing virus (DWV) by injection of virus particles. Adult worker bees were infected with *Nosema apis* and *Nosema ceranae* by the oral uptake of food supplemented with defined spore concentrations. Diseased and control animals were collected at various time points post infection, fixed in formalin and embedded in paraffin. Thin sections of the different body parts were analyzed by fluorescence *in situ* hybridization (FISH) using specific fluorescence dyes labeled oligonucleotide probes for each pathogen and following recently established protocols. *In situ* visualisation of infected cells and tissues will in the end help us to understand the pathogens' life cycles during pathogenesis.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-9**

New findings in genome of *Apis mellifera* filamentous virus

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The honey bee plays an extremely important role as a pollinator of crops and wild plants. Honey bee colony losses noted worldwide since 2006 can heavily impair not only global food production but also ecosystem and biodiversity maintenance. One of the possible causes of this situation is the co-infection of bee colonies with different pathogens including *Nosema apis/ceranae.* This microsporidium is often associated with viruses like Black queen cell virus, Bee virus Y and *Apis mellifera* filamentous virus (*Am*FV). The life span of bees infected with *Nosema* and viruses is shorter than of bees infected with *Nosema* alone.
*Am*FV is a DNA virus. The size of the enveloped particle is 150-450 nm x 150 nm. On the basis of morphological features, *Am*FV was considered to be related to baculoviruses. In 2012 the first fragment of *Am*FV genome was sequenced (822nt long) and submitted to the GeneBank. Phylogenetic analysis of this fragment supports previous assumptions of similarity to baculoviruses.
In our studies we use the Illumina Next Generation Sequencing approach to sequence much longer fragments of genome of *Am*FV. One of the contig that contains the full sequence of previously described BroN gene comprises another gene, which sequence is highly similar to baculoviral ribonucleotide reductase. Other findings about genome structure and possible theories concerning origin of elusive *Am*FV will be presented during the conference.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-10**

Development of prototypes of rapid molecular diagnostic tests for pathogens of honeybees (*Apis mellifera* L.) on chromatographic NALF platform (Nucleic Acid Lateral Flow)

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Honeybees are of inestimable value as agents of cross-pollination and also as workinsect for beekeeping. Honeybee populations have been decreasing globally in recent years because they are affected by environment, human activities, moreover they are susceptible to many pathogens.
Viroses and nosemosis are widespread in honeybees, but despite the serious economic losses they can cause, these are underestimated by the beekeeping industry. An early diagnosis of the causative agents has great importance for the management of the disease and in the establishment of measures to guide therapy and prophylaxis.
We present the development of diagnostic tests based on the NALF (Nucleic Acid Lateral Flow) technology for the detection of the following pathogens of honeybees: Deformed Wing Virus (DWV), Israeli Acute Paralysis Virus (IAPV) and the microsporidian Nosema (*Nosema ceranae*).
DNA and RNA of the pathogen are amplified by isothermal reactions using LAMP (Loop-mediated Isothermal Amplification) in the presence of at least one primer conjugated to Gold Nano Particles (GNPs) that are used to label the molecules of interest. The result of the isothermal reaction is detected by naked eye, in a few minutes, by means of a NALF device.
The assay has the same sensitivity and specificity of a molecular test but being at the same time quicker, cheaper, waste friendly, adapted to basic laboratory equipment and accessible to ordinary technical personnel.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-11**

What Kind of Insects Do You Like?

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Insect constitute the largest and most diverse group of animals on world and also serve as the hosts or nutrient sources. In addition, several insects have a strong influence on people's emotion. To utilize the preference and interest of insects in the field of mental healthcare, a survey study was conducted with individual living in Korea. As results, the most people had a high preference and interest of insect, but some were disagreeable to the insect itself. The preference and interest of insect were high on male, adult and practician experienced insect-related events than female, student and non-practician, respectively. The most favored insects were familiar or pet insects such as *Papilio xuthus*, *Lucanus maculifemoratus, Allomyrina dichotoma* and Lampyridae. These results may be useful to develop a healing program for mental healthcare using insects. Further research is needed to determine the effects of these insect in the mental therapy for this purpose.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-12**

A muscle-infecting microsporidium infecting pink shrimp (*Pandalus montagui*) from Europe: closing in on the type species of *Thelohania*?

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The type species of the genus *Thelohania*, *T. giardi* was described infecting European brown shrimp (*Crangon crangon*) in the late 1800's. Although never rediscovered, recent work describing *T. butleri*, a similarly octosporous microsporidium infecting Canadian pink shrimp (*Pandalus jordani*), provided evidence that *Thelohania* (containing parasites of marine shrimp, freshwater crayfish, and ants) is polyphyletic and in need of significant revision. This work led to proposals that only marine forms should be considered as true members of the genus and that effort should be applied to rediscover the type species. In this study, we describe a novel microsporidium infecting another pandalid shrimp, *P. montagui* from Europe using histological, ultrastructural and phylogenetic data. Although the parasite does not display the characteristic morphological features of *either T. giardi* or *T. butleri* (8 spores contained within each sporophorous vesicle), phylogenetic analysis places it closest to *T. butleri* (91% similarity, 100% coverage of 937bp fragment of SSU rDNA gene) within the broader microspordian tree. Previous work from our laboratory has focussed on the potential for morphological plasticity within Microsporidia infecting the musculature of marine crustaceans. To this end, we propose that despite divergence in form from the type species of *Thelohania*, the close phylogenetic relationship to *T. butleri* suggest that the parasite in *P. montagui* is a species of *Thelohania*. In addition, we provide further evidence that closely related taxa can display wide morphological variance and, that marine thelohanids may display a level of intra-generic plasticity which nullifies the use of morphology in their taxonomy.

FUNGI

Poster / Fungi. Wednesday, 16:30. **FU-1-STU**

Monitoring of entomopathogenic fungi in *Metarhizium* and *Beauveria* treated fields

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Application of entomopathogenic fungal strains for the control of cockchafer grubs was investigated in sour cherry orchards. Safety like possible effect of the inoculum on natural soil microbiota as well as efficacy and fate of these fungi need to be investigated. The applied fungal strains have wide host range, thus we have to determine the risks of their use during repeated long-term applications. Different inoculation methods were compared and the persistence of inoculum was monitored in the soil and on target and non-target organisms. One year after treatments we collected soil samples and grubs from un-treated and treated areas and re-isolated the fungi on selective media. Furthermore we applied PCR analysis for the identification of our *Metarhizium anisopliae* strains. According to *Ya Li & Shuang Hu-Cai* (2011) we used a species-specific primer for the detection of fungus. We were able to detect the presence of *Metarhizium* strains. Neither another entomopathogens (*Beauveria, Lecanicillium*), or other fungi like fusaria gave positive signal with the *Metarhizium*-specific primers. Furthermore, the presence of *M. anisopliae* was detected in about 10 percent of untreated soil samples. It proves that *Metarhizium anisopliae* can be found in the original soil mycobiota, although at a very low frequency.
Research was supported by the grant **GOP-1.1.1-11-2012-0059** „*Development of environment friendly product with the use of entomopathogenic organisms*"..

Poster / Fungi. Wednesday, 16:30. **FU-2**

Distribution of insect-pathogenic soil fungi in agricultural and forest ecosystems in Georgia

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Entomopathogenic fungi naturally occurring in the soil represent a reservoir of antagonists to insect pest. Local strains of such fungi may be adapted to their environment and are of particular interest for usage in biological control. Georgia has a high diversity of altitudes, eco-systems and cropping system and may offer special opportunities for studies of insects pathogens.Soil samples were obtained in 2012-2013 from 8 different geographical sites at different altitudes (600-2200 m a.s.l), representing different agricultural and forest ecosystems, National parks of Georgia. A total 161 soil samples representing 45 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 brongniartii, Metarhizium* spp.*, Lecanicillum sp. Isaria* sp. Also, we isolated *Aspergillus flavus.* The most abundant species was *Beauveria bassiana* (41,4%) and *Metarhizium* sp. (49,4%) from the total number of isolates. Three isolates of both *Metarhizium and- Lecanicillum*

were found, while only one *Beauveria brongniartii*. Interestingly, no entomopathogenic 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. were recovered mostly in agricultural habitats. Our study included a limited number of samples, and more extended studies may reveal additional information about the occurrences of these fungi in different habitats and geographical zones of the South Caucasian region.

Poster / Fungi. Wednesday, 16:30. **FU-3**

Diversity of Entomopathogenic fungi in different citrus cropping systems in Brazil

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Biodiversity studies of entomopathogenic fungi (EF) in agroecosystems are very important to understanding the ecology of indigenous populations, their contributions to pest control and the impact of agricultural practices on their populations. The objective of this study was to investigate the natural occurrence of EF in citrus in São Paulo State, Brazil. Samples were collected in four fields with conventional cropping systems (Santa Barbara D'Oeste, Conchal, Nova Europa and Bebedouro), one organic field (Itirapina) and some abandoned fields in Itapetininga, Anhembi, Conchal, Corumbataí, Limeira and Araras during one year (2013-2014). The EF were isolated from soil samples by selective medium and the "Insect Bait" method using *Tenebrio molitor* larvae, and from pest samples by direct transfer onto PDA medium. The Hypocreales fungi isolated from soil by selective medium were *Metarhizium* sp. (18.9% of 174 soil samples) followed by *Beauveria* sp. (14.3%) and *Isaria fumosorosea* (8%). Using the "Insect Bait" method *Metarhizium* sp. was recovered from 75.9% of the soil samples and *Beauveria* sp. from 1.7% of samples. The insect pests found infected by EF were the citrus snow scale, *Unaspis citri* (Hemiptera: Diaspididae) infected with *Beauveria* sp. and *Pochonia* sp., the sharpshooters (Hemiptera: Cicadellidae) with *Beauveria* sp., the whitefly *Dialeurodes citri*, and citrus blackfly *Alurocanthus woglumi* (Hemiptera: Aleyrodidae) with *Aschersonia* sp., green scale *Coccus virirdis* (Hemiptera: Coccidae) with *Lecanicillium* sp., and two unidentified Lepidopteran with *Cordyceps* sp. in organic and abandoned citrus fields. In the abandoned fields the density of EF in the soil was lower than the conventional and organic fields.

Poster / Fungi. Wednesday, 16:30. **FU-4**

The Entomopathogenic Fungus *Isaria* for Pest Insect Control in Vegetables

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The growing area of vegetables in the EU covers more than 3,000,000 ha. BIOCOMES is an EU funded project to provide fundamental information for the development of plant protection products, based on biocontrol agents (BCA). Currently, the common control of various insect pests is mainly by synthetic insecticides. Nevertheless, several pest insects cause considerable damage in agriculture due to resistance to pesticides.
The aim of the BIOCOMES work package is to develop a new fungal BCA for pest insect control in open field crops and in greenhouses. Presently, we investigate the integration of entomopathogenic fungi into a control strategy. Within different treatments and pre- and post-harvest applications in protected and non-protected cropping systems, we compare the efficacy of at least 10 *Isaria spp.* strains under different laboratory conditions. Moreover, the host range of these strains will be screened, in order to determine the relationship of clade specific differences between virulence and pathogenity factors. Additionally, the effect on beneficial insects like the predatory mite *Typhlodromus pyri* and the seven-spot ladybird, *Coccinella septempunctata,* will be evaluated to assess the possibility for implementation of entomopathogenic fungi in an integrated pest management strategy. As entomopathogenic fungi are known to produce a wide range of secondary metabolites as, e.g., antibiotics or repellents, selected strains will be screened for secondary metabolites and enzyme activities. Actually, first results will be presented.

Poster / Fungi. Wednesday, 16:30. **FU-5**

Prevalence of *Beauveria pseudobassiana* among tick-associated fungal isolates from the Republic of Moldova

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Human and animal disease transmitting hard ticks (Acari: Ixodidae) are of eminent concern for public health and animal farming. Alternatives to tick control by chemical acaricides are highly solicited, and one intensively evaluated biocontrol strategy is based on the use of tick-pathogenic filamentous fungi. An indispensable prerequisite of the development of tick-derived fungal isolates into registered myco-acaricides is their sound taxonomic characterization.
Using a molecular taxonomic approach based on phylogenetic reconstruction from both internal transcribed spacer (ITS) and protein-encoding gene sequences, a set of fungal strains isolated from ixodid ticks in the Republic of Moldova that had previously been assigned to the species *Beauveria bassiana,* together with further tick-derived fungal isolates from different geographic locations in Europe and the North America was characterized at the genus and species level. All fungi investigated were conclusively assigned to one of the two "hyphomycete" genera, *Beauveria* or *Isaria* (Ascomycota; Hypocreales; Cordyci-pitaceae). Within the genus *Isaria*, two species, *Isaria farinosa* and *Isaria fumosorosea*, were equally represented. Within the genus *Beauveria*, the species *Beauveria pseudobassiana* was found to strongly prevail among the isolates from Moldova. In particular, the previous classification as *B. bassiana* could not be confirmed for any of the correspondingly characterized tick-pathogens from Europe and North America. The data presented motivate the hypothesis that within the genus *Beauveria* specific adaptation to ticks might have occurred in the species *B. pseudobassiana*. However, to test this hypothesis, a more extensive molecular taxonomic survey carefully reconsidering previous taxonomic assignments of tick-derived fungal isolates is indispensable.

Poster /Fungi. Wednesday, 16:30. **FU-6**

Diversity and abundance of entomopathogenic fungi on strawberry crops in Brazil

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The aim of this study was to characterize the diversity and abundance of entomopathogenic fungi in arthropods on leaves of strawberry and of spontaneous herbaceous plants from the crop boarders as well as from soil samples of organic and conventional fields in. The aboveground pests were collected from the crop and from the crop border vegetation at four localities of the Minas Gerais state in Brazil and were incubated in high moisture and inspected for fungi, daily. Two methods were used for isolation of entomopathogenic fungi from soil: selective media (SM) and insect baiting (IB) with *Tenebrio molitor*. No entomopathogenic fungi were observed in the aboveground insect pests, while eight mites were infected with *Neozygites floridana*. Pooling all soils samples revealed that *Metarhizium* spp was the most common fungus (73%-SM / 97.9%-IB), followed by *Beauveria* spp (22%-SM / 1.7%-IB) and *Isaria* spp. (5%-SM / 0.4%-IB). Diversity and abundance of entomopathogenic fungi was not much different between organic and conventional fields. For organic cropping alone the following fungi were isolated: *Metarhizium* spp (58.5%-SM / 97.5%-IB), *Beauveria* spp (34%-SM / 2.5%-IB) and *Isaria* spp. (7.5%-SM / 0%-IB) and for crop border vegetation in organic systems *Metarhizium* spp (82.4%-SM / 96.7%-IB), *Beauveria* spp (17.4%-SM / 2.6%-IB) and *Isaria* spp. (0.2-SM / 0.7%-IB). For conventional cropping: *Metarhizium* spp (83.2%-SM / 98%-IB), *Beauveria* spp (16.8%-SM / 1%-IB) and *Isaria* spp. (0%-SM / 1%-IB), and for crop border vegetation around conventional crops: *Metarhizium* spp (86.1%-SM / 100%-IB), *Beauveria* spp (4.3%-SM / 0%-IB) and *Isaria* spp. (9.6%-SM / 0%-IB). The on-going studies on the intra-specific diversity will reveal the role of the crop borders as a reservoir of these generalist natural enemies.

Poster / Fungi. Wednesday, 16:30. **FU-7**

Abundance and diversity of *Metarhizium* spp. in an agricultural landscape in Sweden

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Entomopathogenic fungi belonging to the genus *Metarhizium* are important regulators of insect populations, including agricultural pests, and products based on these fungi have been applied in augmentation biological control of different pest insects. As sustainable agriculture and implementation of integrated pest management is gaining attention, the interest in establishing conservation biological control strategies is also growing. In conservation biological control, habitats or agricultural practices are adjusted to enhance the abundance of resident natural enemies, i.e. the biological control agent. Such approaches require a profound understanding of the control agent's life cycle and its ability to survive in different environments. However, abundance and diversity of these entomopathogenic fungi in Sweden have not been evaluated. In this study, we therefore investigate the occurrence of indigenous *Metarhizium* spp. in transects of a cereal field, a permanent grassland and an unmanaged forest site in Uppland, Sweden using cultivation-dependent techniques as well as quantitative PCR. A collection of new *Metarhizium* isolates from the different habitats will be established, and strains will be characterized by PCR and genotyping. Factors such as soil management and vegetation will be evaluated for their effect on the abundance and diversity of *Metarhizium* spp. This study will generate new information on the potential of using *Metarhizium* for insect pest control in Sweden. Hence, it will facilitate the development of *Metarhizium* based biological control approaches including both augmentation as well as conservation biological control and the use of these approaches in sustainable farming systems in Sweden.

Poster / Fungi. Wednesday, 16:30. **FU-8**

Diversity and distribution of entomopathogenic fungi in Czech Republic soils

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A survey of entomopathogenic fungi was carried out in cultivated and uncultivated soil habitats in Czech Republic. A total of 189 soil samples were collected during October 2013. Two different methods of isolation were employed, selective media containing cicloheximide and SYLLIT 65 WP and *Tenebrio molitor* bait method. Entomopathogenic fungi were detected in all collected soil samples by using selective media, but not with the second isolation method. Eight different taxa belonging to five different genera were encountered by using morphological and molecular identification (ITS and EF 1-α molecular markers). The two more common taxa were unnamed species designated as *Lecanicillium* sp. (14%) and *Metarhizium anisopliae* (44.5%). Additionally, uncultivated soils showed a higher richness in entomopathogenic fungi than cultivated ones.

This is the first time that a monitoring study for the natural occurrence of entomopathogenic fungi was developed covering all Czech Republic. This study constitutes a valuable source for the discovery of indigenous isolates that can be applied in biological control strategies.

Poster / Fungi. Wednesday, 16:30. **FU-9**

Entomopathogenic fungi as plant growth enhancers

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Entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium brunneum*, and *Isaria fumosorosea* are primarily used for managing pests. A preliminary study showed that treating the roots of strawberry transplants with *B. bassiana* significantly promoted its growth compared to untreated plants or those treated with a commercial plant growth enhancer. In another study, soil treatment of strawberry plants with *M. brunneum* appeared to help plants withstand twospotted spider mite (*Tetranychus urticae*) infestations compared to untreated plants. These studies suggest that entomopathogenic fungi could be promoting plant health and growth through mycorrhizal interaction. A study was conducting by soil treatment of potted cabbage plants with various commercial products based on entomopathogenic fungi - *B. bassiana*, *M. brunneum*, *I. fumosorosea*, mycorrhizal fungus - *Rhizophagus irregularis*, and a formulation based on bacterial and fungal combination – *Azorhizobium caulinodans*, *Bacillus subtilis*, *Pseudomonas*

phaseoli, *Rhizobium phaseoli*, and *Trichoderma virens*. Impact of these treatments on plant development will be discussed. Preliminary data show superior growth of cabbage plants treated with *B. bassiana*.

Poster / Fungi. Wednesday, 16:30. **FU-10**

The entomopathogenic fungus *Beauveria bassiana* improves the growth of *Triticum aestivum* and *Triticum durum*

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The main role of Entomopathogenic Fungi (EF) is to kill insect. However, it was recently discovered that many EF, especially hypocrealean ascomycetes, have additional not fully understood ecological roles. This research deals with the effect that EF have on growth, nutritional status, and hormone levels of inoculated plants, *Triticum aestivum* and *Triticum durum*. Three inoculation methods were used using a conidial suspension of *B. bassiana* (Balsamo) Vuill with a concentration of 10^8 conidia mL^{-1}, soil treatment, seed dressing and leaf spraying (2 first leaves of wheat plants 7 days after germination), with 25 plants per treatment either treated / inoculated or control. Plant growth parameters were determined and evolution of the fungal inoculum in the soil and colonisation of plant tissues (leaves and roots) assessed through re-isolation of *B. bassiana* at different phenological states. The fungus was revealed to be rhizosphere-competent, with root re-isolation percentages ranging from 20 to 80% for plants grown on soil treatment and seed dressing. Percentage of fungal re-isolation from leaf tissues was significantly higher in plants inoculated by leaf spraying ranging between 8 and 75 %. At the end of the crops, it was detected that the dry weight, the total root length, the quantity of some nutrients and yield of inoculated plants was higher than in control plants. The possible origin of these differences in *B. bassiana* inoculated plants and their implications for pest and disease control and the promotion of plant growth are being investigated.

Poster / Fungi. Wednesday, 16:30. **FU-11-STU**

Interactions between cowpea plants vs. *Metarhizium* spp. entomopathogenic fungi

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In recent years, *Metarhizium* spp. fungi have been reported to associate with plants through rhizosphere competence and endophytic growth. Benefits to both the fungus and the plant, at least in some cases, is beneficial to both members of the pair. In the present study, germination of two important *Metarhizium* species was analyzed after incubation of conidia on young plant leaves. Seeds of *Vigna unguiculata* (cowpea) were planted in sterile soil and incubated with ambient light at room temperature for 10 days. Aqueous suspensions of *Metarhizium robertsii* ARSEF 2575 or *Metarhizium brunneum* ARSEF 1095 were brushed directly onto plant leaves. Control plants, to ensure conidial germination, had their leaves brushed with potato dextrose agar (PDA) plus 0.05% chloramphenicol and 0.002% benomyl; air dried; then the fungus suspension was brushed on the leaves. After 24h and 48h, 0.5 cm^2 leaf pieces were examined by scanning electron microscopy (SEM). Conidia of both fungal isolates germinated on cowpea leaves 24h and 48h after inoculation on both PDA treated (control) and PDA not treated (test) leaves. SEM observation showed conidial adherence but with no preferred attachment sites. Each conidium produced one germ tube; and both long and short germ tubes were observed. Their growth over the plant cuticle was random (had no apparent targets). There was no evidence of appressorium formation. However, some *M. brunneum* ARSEF 1095 conidia that germinated on non-PDA-treated leaves had images that suggested direct penetration. Culture studies with surface-sterilized fungus-exposed leaves are underway to verify or deny cuticular penetration.

Poster / Fungi. Wednesday, 16:30. **FU-12**

Biological control in oilseed rape: An attempt to establish the entomopathogenic fungus *Beauveria bassiana* as an endophyte in oilseed rape plants

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With the rapid spreading of the cultivation of oilseed rape (*Brassica napus* L.), the populations of pest insects of rapeseed also increase, in particular the rapeseed pollen beetle (*Brassicogethes aeneus*) and rape stem weevil (*Ceutorhynchus napi*). Hence, the aim of the investigations within the scope of biological control is to establish the entomopathogenic fungus *Beauveria bassiana* Naturalis ATCC74040 as a systemic endophyte in oilseed rape. Blastospores of *B. bassiana* (10^5 Sp/ml) from Czapek liquid medium were infiltrated into rape leaves. The plants were held with 80% RH and 20°C on long day conditions. Between 3 days and 4 weeks leave samples were taken and examined by fluorescence-microscopy, either with Blankophor or specifically with polyclonal primary antibodies against *B. bassiana*. PCR primers targeting a characteristic partial sequence of a self splicing group-I intron within the 28S rRNA encoding gene of *B. bassiana* Naturalis ATCC74040 were designed and used for strain-specific diagnosis. While the fungus was found to be persistent on the epidermis, only few hyphae could be detected microscopically in intercellular space of the leaves. By means of PCR, *B. bassiana* Naturalis could be proven successfully in rape tissue samples; a clear molecular proof of systemic growth within leaves is still pending. Possible defense mechanisms are discussed.

Poster / Fungi. Wednesday, 16:30. **FU-13**

Azygo- and zygospore formation of *Neozygites floridana* in the two-spotted spider mite (*Tetranychus urticae*) in strains from tropical and temperate regions

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Neozygites floridana is an obligate fungal pathogen of mites in the family Tetranychidae and is an important natural enemy of the two-spotted spider mite (*Tetranychus urticae*). Until now, information about the formation of azygospores remained to be fully confirmed. In this study, we document the formation of

azygospores by a Brazilian *N. floridana* strain and the formation of azygospores and zygospores by a Norwegian *N. floridana* strain both in the host *T. urticae*. Evidence of both zygosporogenesis and azygosporogenesis was also found in the same individual in the Norwegian stains. Further we report the presence of immature azygospores with 1-3 nuclei for the Norwegian strains, immature resting spores (probably azygospores) with 1-8 nuclei for the Brazilian strain, and mature resting spores with 2 nuclei for both the Norwegian and the Brazilian strains (azygo- or zygospores). Our observations suggest that the immature resting spore (prespore) of both strains begins in a multinucleate condition but that the nuclear number is reduced during maturation until mature resting spore is binucleate regardless of its origin as zygospore or azygospore.

Poster / Fungi. Wednesday, 16:30. **FU-14**

Susceptibility of *Biomphalaria glabrata* egg masses to fungal infection

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Aquatic *Biomphalaria glabrata* snails from the neotropics are most common in stagnant or slow-flowing water habitats. Quantities of egg masses are laid near the water surface on submerged substrates but are often eventually exposed to desiccation and natural enemies. Almost nothing is known about fungal pathogens acting against these snails. We report on the ovicidal activity of *Metarhizium anisopliae* (IP 46) and *Beauveria bassiana* (ARSEF 9588). Freshly laid egg masses (5 masses each test of four independent repetitions) were either exposed to water and treated with 2x10e7 conidia or hyphal bodies/ml of these fungi or treated topically (2x10e7 conidia or hyphal bodies) and then incubated in a permanent water film in a moist chamber at 25ºC. Controls were treated with water only. Egg masses were checked daily for fungal growth and eclosion of juveniles. After application of conidia or hyphal bodies, IP 46 developed distinct mycelium and new conidia on egg masses in water film, and hyphal bodies yielded no later eclosion of juveniles. No mycelium developed when ARSEF 9588 was applied to egg masses exposed in water films and all juveniles eclosed. In water, both fungi developed mycelium after application of conidia or hyphal bodies to egg masses, and juveniles failed to eclose. All juveniles eclosed from uninoculated egg masses exposed in water or film. The results suggest that both *M. anisopliae* and *B. bassiana* may act against *B. glabrata* egg masses, but that the degree of molluscicidal activity depends on the type of fungal inoculum applied.

Poster / Fungi. Wednesday, 16:30. **FU-15**

Antimicrobial, Antioxidant and Anticancer Activity of Culture Filtrates from Entomopathogenic Fungi

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Entomopathogenic fungi are natural pathogens of insects and contribute to the regulation of host insect populations in the environment. Several these fungi produce a wide range of secreted enzymes, secreted protein toxins and secondary metabolites to overcome host defenses and ultimately kill the host, and to defend host resources against competing pathogens and saprophytes. Therefore, this study was performed to select the antimicrobial activity of entomopathogenic fungi form Korea soils against plant pathogenic bacterium *Ralstonia solanacearum* and plant pathogenic fungus *Botrytis cinerea* using dual culture technique on SDYA. In addition, we also performed to screening of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity compounds from liquid culture filtrates of entomopathogenic fungi and investigate to it's anticancer activity. As results, 12 isolates, 6 isolates and 25 isolates showing of these fungal metabolites produced antibacterial, antifungal and radicals scavenging activity compounds, respectively. The preferential antimicrobial, radical scavenging and anticancer activities give evidence that these entomopathogenic fungal metabolites might be useful as a source for plant pathogen control and pharmaceutical interests.

Poster / Fungi. Wednesday, 16:30. **FU-16**

Evolutionary-ecological strategies of *Metarhizium robertsii*

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The species of the entomopathogenic fungus *Metarhizium* include forms characterized by different pathogenic strategies. Two strains of entomopathogenic fungus *M. robertsii* with different strategies were investigated. The strain Mak-1 («growth strategy») is characterized by slow killing speed of different insect species (Orthoptera, Coleoptera, Diptera, Lepidoptera) and abundant sporulation on cadavers. The strain P-72 («toxin strategy») is characterized by significantly rapid killing speed, but sporulation of this strain was detected only on Lepidoptera. Thus the fungi specialization can be associated with necrotrophic (but not biotrophic) phase of life cycle. In addition P-72 is characterized by the higher level of destruxin B, E production, rapid activation of conidia on the artificial media and insect's cuticle. The strain P-72 was more productive in media from plant compounds while Mak-1 - on insects and media of them. Our results show that «non- toxigenic» strain has higher adaptation to entomoparasitic nutrition, and the «toxigenic» strain to saprophytic nutrition. We found the change of the defense systems of Colorado potato beetle (*Leptinotarsa decemlineata*) larva (increasing of phenoloxidase in cuticle and detoxificative enzyme in fat body and hemolymph, decreased rate of cells immunity) under infection by the «toxigenic» strain but not by strain with «growth strategy». Our data support hypothesis that evolution of entomopathogenic fungi *Metarhizium* was directed with a loss association with plants and formation of specialized entomoparasitic forms.

Poster / Fungi. Wednesday, 16:30. **FU-17**

Mycelial and conidial thermotolerance of *Metarhizium anisopliae s.l.* IP 46 and *Metarhizium robertsii* ARSEF *2575*

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High temperature is a very important environmental stressor that may limit efficacy of fungi in arthropod biocontrol programs; however, formulation of fungal propagules is suggested for increasing performance of fungi. The current study was designed to evaluate the radial growth of colonies of *Metarhizium anisopliae* s.l. IP 46 and *M. robertsii* ARSEF 2575 on PDAY

culture medium incubated at 27±1ºC (optimum) or 32±0.5°C (heat stress) for 15 days. Colonies diameter was measured daily, and at day 15 the conidia produced were quantified, and their viability assessed. In addition, thermotolerance of conidia prepared in different additives was investigated; accordingly, dried conidia were suspended in water solution (Tween 80, 0.01%), commercial emulsifiable or non-emulsifiable oils or carboxymethyl-cellulose gel (CMC), and exposed to 45±0.5ºC for 4, 6 or 8h. Germination was assessed 48h after inoculation of conidia onto PDAY plates. A significant reduced radial growth and conidial production were shown in colonies incubated at 32±0.5°C, but conidial viability was high (>98%) for both fungi grown under optimum or heat-stressed conditions. Viability of conidia suspended in water solution, commercial emulsifiable oils or CMC, and exposed to 45±0.5ºC was drastically low [0% mean relative germination (RG) at 8h exposure]. Conversely, conidia suspended in non-emulsifiable canola or mineral oil had high viability (69.3% and 71.8% RG for ARSEF 2575, and 95.0% and 80.2% RG for IP 46, respectively, both at 8h exposure). In conclusion, oil formulation minimizes the effects of high temperature to conidia of these entomopathogenic fungi, indicating that conidia applied to the field could persist longer in heat-stressed environments and that their development may occur during periods reaching optimum temperatures.

Poster / Fungi. Wednesday, 16:30. **FU-18**

Delayed germination of heat-stressed conidia of *Metarhizium anisopliae* on tick cuticle

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The current study assessed the germination of heat-stressed conidia of *Metarhizium anisopliae* IP 119 on cuticle of *Rhipicephalus sanguineus*. Aqueous conidial suspensions (Tween 80, 0.01%) of *M. anisopliae* IP119 were exposed to 0 h (non-heated control) or 4 h at 45±0.5°C (heat-treated test) in a water bath, and then inoculated onto either the dorsal surface of *R. sanguineus* engorged females or onto PDAY culture medium. The samples were incubated at 27±1°C and RH>80% for 0, 12, 18, 24, 36, 48 or 72 h. After each incubation time, ticks were dissected, i.e., the dorsal cuticle was removed and immersed in Calcofluor White 2% overnight, then observed by fluorescence microscopy for evaluation of conidial germination. Conidial germination on PDAY plates was assessed using a phase-contrast microscope. A minimum of 300 conidia per cuticle or plate was evaluated, and percent germination calculated. It was found that conidial germination on tick cuticle was delayed in comparison to germination on artificial culture medium, regardless the incubation time. When conidia were exposed to heat, a higher percent germination was detected on PDAY (61.5%) in comparison to the tick cuticle (13%) at 72 h after inoculation. On tick cuticle, appressoria from non-heated (control) conidia were observed 36 h after inoculation, whereas no appressoria were seen from heated conidia (test) at any incubation period after inoculation, including 72 h. In conclusion, heated conidia germinated faster when they were inoculated on PDAY than when they were applied to the tick cuticle. This result suggests that the negative effect of heat on conidial germination was greater when the conidia were applied to arthropod cuticle than would be predicted by *in vitro* (artificial medium) thermotolerance tests. In addition, the technique of fluorescence microscopy proved to be a simple method for visualizing germinated conidia and appressoria on the cuticle of *R. sanguineus*.

Poster / Fungi. Wednesday, 16:30. **FU-19**

Influence of environmental factors on insects resistance to anamorphic fungi

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We studied effect of different factors such as suboptimal temperatures, sublethal bacterial infection (*Bacillus thuringiensis*), synthetically and natural insecticides (pirimiphos-methyl, *Cordyceps militaris*) and venom of parasitoid *Habrobracon hebetor* on defense systems of wax moth *Galleria mellonella* and Colorado potato beetle *Leptinotarsa decemlineata.* Moreover insect susceptibility to fungi *Beauveria bassiana* and *Metarhisium robertsii* under these factors has been examined. We found the decreasing of phenoloxidase activity in hemolymph and cuticle, and detoxicative enzymes activity (nonspecific esterases, glutation-S-transferases) in hemolymph, as well as in encapsulation response. Thus dramatic depression in host's defense systems led to increased susceptibility of insects to fungi from ten to several thousand times. Our data support hypothesis that low specificity of anamorphic entomopathogenic fungi is closely associated with their ability to infect insects with defense system seriously suppressed by various environmental factors.

Poster / Fungi. Wednesday, 16:30. **FU-20**

Intraspecific and interspecific variation in osmotolerance of entomopathogenic fungi

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Entomopathogenic fungi must be capable of cell division under multiple stresses imposed during the various stages of the lifecycle, some of which take place on the insect surface or within the hemolymph. These include the energy-expensive synthesis and retention of compatible solutes to maintain osmotic pressure. The windows for osmotolerance of 24 isolates of entomopathogenic fungi were determined by assessing conidial germination over a range of KCl concentrations. Germination was evaluated on potato dextrose agar (PDA; control) or PDA+KCl using 31 concentrations of KCl from 100 to 3000 mM (after 24 h; 26 °C). *Trichothecium roseum* was the most osmotolerant (≤ 3000 mM KCl), followed by *Lecanicillium aphanocladii*, *Simplicillium lanosoniveum*, and *Isaria fumosorosea*. Several fungal species showed moderate osmotolerance (≤1700 mM) including *Metarhizium robertsii* (for some isolates), *Metarhizium brunneum*, *Metarhizium anisopliae*, *Tolypocladium inflatum, Tolypocladium cylindrosporum*, and *Fusarium coccophilum*. Some isolates showed modest levels of osmotolerance (≤ 1400 mM), including one isolate of *M. robertsii*, one of *M. anisopliae*, two of *M. acridum*, and *Beauveria bassiana. Aschersonia aleyrodis* and one isolate of *M. brunneum* were relatively intolerant to osmotic stress (≤ 1000 mM KCl). These findings indicate high levels of inter- and intraspecific variability in osmotolerance for insect-pathogenic fungi. Eighty percent of *Trichothecium roseum* conidia germinated at 2000 mM KCl (equivalent to 0.928 water activity), with a LC50 at 2300 mM, and some germination at < 0.890 water activity (on 3000 mM KCl). This suggests that *T. roseum* is highly xerotolerant and may therefore be unique amongst the entomopathogenic fungi.

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Poster / Fungi. Wednesday, 16:30. **FU-21**

Different intensities of visible light during mycelial growth induce differently the conidial tolerance to menadione in *Metarhizium robertsii*.

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The threshold of illumination during mycelial growth influenced the conidial tolerance of an entomopathogenic fungus to the oxidative agent menadione. *Metarhizium robertsii (*ARSEF 2575) was grown at 26 °C for 14 days in five treatments: 1) minimal medium (MM) in the dark; 2) potato dextrose agar (PDA) in the dark inside the Panasonic incubator; 3) PDA medium under continuous visible light in the Panasonic incubator; 4) PDA medium in the dark inside the Marconi incubator; 5) PDA medium under continuous visible light inside the Marconi incubator. For the Panasonic incubator, three intensities of light were studied with 1, 3, and 5 lumens. The germination of conidia produced under these treatments was subsequently evaluated on PDA medium supplemented with menadione at the concentrations 0.10 and 0.15 mM. For control, conidia germinated on PDA medium. The germination was evaluated counting at least 300 conidia after 24 h at 26 °C. Each treatment was repeated four times with a new batch of conidia produced for each repetition. Conidia produced on minimal medium were more tolerant to menadione, followed by conidia produced under visible light inside the Marconi incubator. Conidia produced inside the Panasonic incubator at 5 lumens were more tolerant to menadione, but less tolerant than conidia produced under light in the Marconi incubator. Conidia produced in the Panasonic incubator at 1 and 3 lumens showed somewhat increased tolerance as compared with control in the dark. Therefore, growth under visible light produced conidia more tolerant to menadione.

Poster / Fungi. Wednesday, 16:30. **FU-22**

Effect of *Metarhizium* spp. growth media on the accumulation of destruxins in a 10-L stirred tank reactor

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Entomopathogenic fungi secrete a wide range of metabolites, mostly products of secondary metabolism. These metabolites serve different functions depending on the ecological niche of the fungus. Nevertheless, the EU-approach to microbial metabolites is still under discussion and therefore, three *Metarhizium brunneum* production strains were assessed for their secondary metabolite secretion (i.e. destruxin A, B and E) in a 10-L stirred tank reactor. Biomass production on the Sabourand-4 glucose - complete medium - and on a modified Czabek-Dox media, blended with yeast extract without peptone was tested two-times in batch-fermentation runs. The aim was to figure out whether secondary metabolite impurities (i.e. destruxin analytes) in the technical BCA products derive from a overdosage of complex nutrient ingredients or if they are routinely formed during the BCA production process. The destruxin A, B and E accumulation considerably decreased for all three production strains by avoiding peptone as nitrogen source. Comparing the three production strains in both culture broth batch-systems it must be concluded that the strains differ in the amount of destruxin accumulation. Crude extract products are now available for the purpose of further risk assessment studies of *Metarhizium* metabolites (a.o. cytotoxicity and genotoxicity studies).

Poster / Fungi. Wednesday, 16:30**.** **FU-23**

Evaluation of destruxin A production in four strains of *Metarhizium* by capillary electrophoresis

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Destruxin A (dtx A) is one of the main secondary metabolites produced by *Metarhizium* strains that exhibit insecticidal activity. Due to its toxicity, and the fact that it could be a risk to humans and the environment since it is able to enter in the food chain, the interest in learning more about the detection of this metabolite has increased in recent years. In this study the production of dtx A by four different strains (BIPESCO5, EAMa 01/58-Su, ART 2825 and ARSEF 23) was evaluated. These strains were grown in four different culture mediums (CM: semi-synthetic complete medium; MM: minimal medium; OSM: osmotic stress medium; CN2: peptone in water). All analyses were carried out using a powerful separation technique named Capillary Electrophoresis with Ultraviolet detection (CE-UV). The results showed that ARSEF 23 cultivated in MM medium was the only strain which produced dtx A with a maximum concentration of 20.2 mg/L. In CM medium, BIPESCO5, ARSEF 23 and EAMa 01/58-su strains produced dtx A at different concentrations (24.4 mg/L, 9.9 mg/L and 7.8 mg/L, respectively). Under the CE conditions selected, dtxA was not detected in ART 2825 strain. No strains cultivated in either OSM or CN2 medium produced detectable amounts of dtxA. Our results indicate that the production of dtx A by strains depends on the culture medium, probably related to glucose content. Additionally, it can be confirmed that CE coupled with UV detector is a suitable tool to identify and quantify dtx A (at concentrations higher than 0.5 mg/L) in fungal culture medium.

Poster / Fungi. Wednesday, 16:30. **FU-24**

Entomopathogenic fungal genera and the 1F=1N standard: The shape of the future begins to emerge

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Recent changes to the International Code of Nomenclature for algae, fungi and plants compel changes in how pleomorphic fungal genera are named, and they disallow the retention of separate generic names for sexual and asexual morphs of the same taxa. These changes broadly affect many fungi, but

strongly affect the taxonomically complex insect pathogens of Hypocreales. Molecular data and phylogenetic reconstructions are being used to develop community-driven, consensus-based proposals for conservation and rejection of generic names for the affected fungi. These efforts seek to stabilize generic concepts around well supported monophyletic clades while minimizing disruption to the diverse research and user communities dealing with these fungi. Inevitably, some widely studied genera will be synonomized, and their names will no longer be available except in a descriptive manner (e.g., hirsutelloid morphology rather than *Hirsutella*). Proposals for Ophiocordycipitaceae and some taxa in Clavicipitaceae (notably *Metarhizium* and closely related genera) are now available. The current draft proposal for genera of Cordycipitaceae is presented here. Despite the substantial effort involved in generating the lists presented here, real challenges in resolving some relationships remain; future studies can be expected to justify the recognition of still more segregate genera than are now listed in the proposals. The senior author will continue to update the SIP membership about relevant changes at future SIP meetings and the ARSEF collection's website (http://www.ars.usda.gov/Main/docs.htm?docid=12125).

Poster / Fungi. Wednesday, 16:30. **FU-25**

Genotyping of Georgian isolates of entomopathogenic fungi *Beauveria* spp.

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Our research is about genotyping different subspecies isolates of *Beauveria* collected from various regions of Georgia. *Beauveria* spp is one of the most widely spread entomopathogenic fungi in agriculture. It is a producent of toxins and biological active materials, which can cause high mortality in different species of pests. Nowadays, there is high interest towards active strains of *Beauveria.* Use of molecular biology techniques has demonstrated that *Beauveria* spp (7 isolates from different habitats and geographical zones of Georgia) unites unknown species and their determination by traditional conidial morphology is impossible.We have done phylogenetic characterization of *Beauveria Bassiana*: (I) Polymerase Chain reaction (PCR) to differentiate the clades of Georgian strains (It has never been investigated to which clades A, B or C they belong); (II) Sequencing of DNA fragments from ITS region (the rRNA gene cluster) and (the Elongation Factor 1-alpha) EF1 and (the intergenic) Bloc region. At present, we plan to identify proteins that are responsible for the virulence of Beauveria Bassiana. This study gives us opportunity to understand population of Beauveria and its future applications in effective biocontrol strategy of pathogens. Attention to biocontrol is a breath taking perspectives for sustainable development of the world.

Poster / Fungi. Wednesday, 16:30. **FU-26**

Genetic characterization, fungicide sensitivity, and aphicidal potential of *Lecanicillium* fungi from Argentina

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Mitosporic fungi of the genus *Lecanicillium* (Ascomycota; Hypocreales) are of particular interest as biological control agents for phloem-sucking plant pests including aphids. Bioprospection for these fungi in Argentina has given rise to a set of single-spore derived *Lecanicillium* strains isolated from a wide range of original hosts. Current species delineation within the taxonomic genus *Lecanicillium* that consists of the three "core species" *Lecanicillium lecanii, L. muscarium*, and *L. longisporum* as well as further less closely related species, is not free of ambiguity. For species-level characterization of *Lecanicillium* isolates, a set of five genetic markers comprising one mitochondrial (NMS) and two nuclear (ITS, IGS) ribosomal RNA operon together with one mitochondrial (*nad1*) and one nuclear (*ef1α*) protein-encoding sequences, has been employed. The aggregated information from these markers indicates that fungal isolates from Argentina mainly, but not exclusively belong to the *Lecanicillium* core species. Moreover, the set of *Lecanicillium* strains has been investigated for fungicide sensitivity. Between strain differences in susceptibilities have been found to be important and not necessarily in line with systematics, making careful determination of sensitivity to agriculturally used fungicides an important criterion of biocontrol agent selection. However, the fungicidal polyketide compound soraphen has been found of outstanding activity against a wide variety of isolates from all species investigated. On the basis of these results, a subset of strains has been selected for virulence bioassays against the green peach aphid, *Myzus persicae*, an important agricultural pest in Argentina and other parts of the world.

Poster / Fungi. Wednesday, 16:30. **FU-27**

Species-specific PCR assay to identify and discriminate *M. pingshaense*, *M. anisopliae*, *M. brunneum,* and *M. robertsii*

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Metarhizium comprises important fungal pathogens of insects and several species in the *M. anisopliae* complex are in use for biological control of insect pests. The most recent taxonomic revision of the *M. anisopliae* species complex used a multilocus phylogenetic (EF-1α, RPB1, RPB2 & β-tubulin) approach and nine species are now recognized. Accurate molecular identification of these species is possible using the 5' region of EF-1α or one of seven recently developed nuclear intergenic loci. The goal of this study was to develop a species-specific PCR assay to rapidly identify species of the "PARB" clade, which includes *M. pingshaense*, *M. anisopliae*, *M. robertsii* and *M. brunneum*, without the need to obtain full-length sequence reads. Markers included in the recent multilocus phylogeny (ITS, rIGS, EF1-α, EF1-5', RPB1, RPB2 and β-tubulin) and 5 nuclear intergenic (nucIGS) sequence markers for *Metarhizium* were screened for the presence of species-specific sequence signatures amenable for discriminatory PCR primer design. One primer pair was designed each for *M. anisopliae* (rIGS), *M. robertsii* (rIGS) and *M. pingshaense* (MzIGS2), and two primer pairs were designed for *M. brunneum* (both MzIGS2). Specificity

of the different primer pairs was tested by performing BLAST similarity searches and PCR amplifications on a collection of 65 strains representing 11 different *Metarhizium* species. The approach was further validated by identifying soil isolates collected from a Swiss meadow.

Poster / Fungi. Wednesday, 16:30. **FU-28**

Species identification of entomopathogenic fungi of the genus *Lecanicillium* (=*Verticillium lecanii* s.l.) by mitochondrial gene sequences

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For species identification of entomopathogenic fungi of the genus *Lecanicillium* (former *Verticillium lecanii* Zimm. Viegas) from collection of All-Russian Institute of Plant Protection, sequencing of mitochondrial gene nad1 was exploited. Among 39 isolates, 36 showed attribution to *Lecanicillium muscarium*, 2 – to *Lecanicillium psalliotae* and one – to *Lecanicillium longisporum*. In *Lecanicillium muscarium,* 4 nad1 molecular haplotypes were detected. Only one of them was identical to that already present in Genbank (EF512920). Two novel haplotypes were 99.3-99.7% similar to each other and to the former haplotype. Finally, the fourth haplotype was similar to the other three at the level of 97.9% sequence similarity and was represented by 14% of the isolates under study. The geographic origin and isolation source (partially reflecting the host specificity) were diverse with no consistent pattern among haplotypes. Supported by RFBR # 13-04-01905.

Poster / Fungi. Wednesday, 16:30. **FU-29**

The genomic basis for evolved resistance to *Beauveria bassiana* in *Drosophila melanogaster*

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We use an “evolve and resequence” approach to determine the genomic basis for evolved resistance to the fungal pathogen *Beauvaria bassiana* in the genetic model insect, *Drosophila melanogaster*. Entomopathogenic fungi, such as *B. bassiana*, are used in biological control of mosquito vectors of dengue fever and malaria, and of various agricultural insect pests. To better understand mechanisms of insect resistance to *B. bassiana*, we artificially select *D. melanogaster* for increased resistance to this pathogen in very large, replicated experimental populations. The populations that are selected for increased resistance to *B. bassiana* have not evolved cross-resistance to bacterial pathogens, which suggests that selection may be acting on mechanisms outside of core immunity. We genotype the selected and control populations at multiple generations throughout selection to identify relevant genes and to make inferences about the temporal trajectories of adaptive alleles. We are developing novel methods for analysis of pooled sequences from such evolve and resequence datasets that will provide better assessment of technical artifacts and accurately identify regions of the genome that have responded to selection.

Poster / Fungi. Wednesday, 16:30. **FU-30-STU**

Behavioral control of malarial mosquito by entomopathogenic fungi: Death as the vector

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Our previous study clarified infection of entomopathogenic fungi via the proboscis route is important on rapid mosquito death rather than infection route from tarsomere, and death of mosquito highly correlated with fungal invasion to brain. We developed a hypothesis that fungal infection via proboscis route can affect to mosquito behavior, and the aim of this study was to investigate the alteration of host searching behavior of mosquito by entomopathogenic fungi infection until the mosquito dies.

The mosquitoes were inoculated with *B. bassiana s.l.* 60-2, and quantification of the total amount of host searching behavior in a free flight system by using automated-recording device was conducted. Attractiveness of fungus infected mosquitoes and mock mosquitoes to the heat (40°C) and the color (black) were evaluated in this device for 10 days. As a result, attractiveness to the heat was drastically decreased from 3 days post inoculation, whereas attractiveness to the color has a tendency to decrease from 6 days post inoculation. This reduction of response to mosquito attractant might be caused by fungal infection to their head where has various important sense organ to search host. It will inhibit or danage to their heat and visual sensors or sensory neuron, then mosquitoes became less able to recognize host cues (death as the vector). Although conventional vector control has only focused on killing vectors, our results indicate that there need holistic evaluation as disease transmission risk on vector control using entomopathogenic fungi including lethal and sub-lethal effects.

Poster / Fungi. Wednesday, 16:30. **FU-31**

Effect of *Metarhizium brunneum* strain LRC112 and *M. anisopliae* F52 on non-target Carabid Beetles

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Carabid beetles are considered to be among the most important beneficial insects in agricultural ecosystems and are commonly associated with agricultural fields in British Columbia. Sustainable treatment to control click beetles and wireworms should have little impact on non-target carabid beetle populations. In the present study, we examined the effect of the *M. brunneum* strain LRC112 on adult *Agriotes obscurus* and on common local Carabid species. Further, we compared the effects of *M. brunneum* strain LRC112 to the commercial *M. anisopliae* F52 strain. Examined Carabid beetle species were less susceptible to the tested *Metarhizium* strains than *A. obscurus* beetles. Additional assays at multiple spore concentrations of both *Metarhizium* strains were conducted on two common Carabid species: *Pterostichus melanarius* and *Calathus fuscipes*. For both beetle species, significant mortality was observed at the highest *M. anisopliae* F52 concentration, whereas little mortality was observed at the highest *M. brunneum* LRC112 concentration.

Poster / Fungi. Wednesday, 16:30. **FU-32**

Effect of a local strain of the fungus against *Corythucha ciliata* (Say) and *Glyphodes pyloalis* (Walker) in Georgia

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The sycamore lace bug, *C. ciliata* is one of the most destructive pest of plane trees (*Platanus* spp.) all over the world. This pest is also known to be major nuisances in Georgia since plane trees has been very popular in parks and planting of the cities. The lesser mulberry pyralid, *G. pyloalis* which was spread and caused damage to *Morus alba* in recent years in Georgia is very big problem as well.
Isaria fumosorosea isolated from pupae of *Hyphantria cunea* Drury in Georgia was evaluated to determine its potential as a biological control agent of these pests. Second and third instar larvae of *G. pyloalis* were collected in Tbilisi from *Morus alba* trees and *C. ciliata* adults were collected from the bark of *Platanus* trees in Kutaisi, Georgia. A conidial suspension, concentration 10^9 conidia/ml, was used for both experiments. The suspension was applied to bark to expose *C. ciliata* adults and *M. alba* leaves to expose *G. pyloalis* larvae under laboratory conditions. Efficacy, corrected with mortality in the control treatment, was calculated according Schneider-Orelli's formula. *I. fumosorosea* showed 30% corrected efficacy against larvae of *G. pyloalis*, and 50% for *C. ciliata*. The results of this study suggest that larvae of *G. pyloalis* were tolerant to the induced mycoses caused by *I. fumosorosea,* but more effect on the mortality of *C. cilliata* adults. Experiments are needed to determine the IC_{50} of the fungus for the two pests and to develop appropriate application methods if efficacy proves to be sufficient.

Poster / Fungi. Wednesday, 16:30. **FU-33**

The effect of pesticides used in strawberry and soybean on the mite pathogenic fungus *Neozygites floridana*

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Neozygites floridana is an important natural enemy of the two-spotted spider mite, *Tetranychus urticae*. Pesticides used in strawberry and soybean that might affect the conservation and enhancement of this beneficial fungus in Integrated Pest Management (IPM) systems were therefore studied in laboratory. Eighteen pesticides were sprayed on mummified mites killed by the *N. flordana* isolate ESALQ1420 placed on coverslips with alphanumeric coded squares. The effect of these pesticides on the sporulation and capilliconidia production (germination) of *N. floridana* were determined. Recommended concentrations (RC) and half of this concentration (RC/2) were used, and the control was sprayed with distilled water plus 0.05 % Tween 80. The treated cadavers were set to sporulate for 12h in darkness at 25±2°C and 100% RH. The acaricide Vertimec (Abamectin) at half dose resulted in a primary conidia production of 1283(±169) and 38%(±12) of these produced capilliconidia (germinated). RC of Folicur (Tebuconazol) resulted in a primary conidia production of 1558(±308) and 37%(±11) germination. Further, RC of the insecticide Danimen (Fenpropatrin), resulted in a primary conidia production of 1057 (± 201) and a resulting 37%(±11) germination. RC of Talcord (Permethrin) resulted in 1292 (±335) primary conidia and 74%(±4) germination and Karate at RC/2 in 2985(± 337) primary conidia and 83%(±) germination. This demonstrates that Vertimec, Folicur, Danimen, Talcord and Karate (Lambda-cyhalothrin) were the five pesticides that had the lowest impact on *N. floridana*. Products containing sulfur even in RC/2 were detrimental to *N. floridana*. Thiovit Jet (sulfur) resulted in a primary conidia production of only 162(±84) and 0%(±0) germination and no sporulation was observed from mummified mites sprayed with Kumulus (sulfur). These results are important considering that organic farmers extensively use sulfur-based products in order to control phytopathogenic fungi.

Poster / Fungi. Wednesday, 16:30. **FU-34**

Development of a granular formulation of *Metarhizium brunneum* based on mycelial fragments

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The application of the entomopathogenic fungus *Metarhizium brunneum* strain Ma43 (=BIPESCO 5 = F52) against soil dwelling pests like *Otiorhynchus sulcatus* needs specific requirements on the product. Although the fungus can be grown on solid media the fermentation time in solid state fermenter is long and labor intensive. Additionally, problems with the application of the fermented grain are reported. Therefore, we investigated the possibility of formulating mycelial fragments. Mycelium of Ma 43 was produced in a liquid fermenter and was homogenized to get a flowable suspension. The results demonstrate that humid heat of up to 70° C reduce the viability of the mycelial fragments whereas dry heat of up to 70° C did not influence the viability. Further experiments on fluid bed drying demonstrated that mycelial fragments can be coated on millet at temperatures of 50° C. After coating the fungus was growing and sporulating on the surface of the millet under humid conditions. Protectants like lactose enhanced the viability after fluid bed drying. Further optimization steps and the practicability of mycelial fragments based formulations will be discussed.

Poster / Fungi. Wednesday, 16:30. **FU-35**

Innovative biological products for soil pest control: Outline of an EU project

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Many herbivorous insect pests have soil dwelling larval stages, which are difficult to control. These subterranean insect pests, such as the western corn rootworm, wireworms, black wine weevil, sciarids, white grubs, and tipulids, currently need to be controlled by insecticidal applications. However, in complying with EU directives, several pesticides are or will be phased out in the near future, requesting new and complementary control

strategies. INBIOSOIL explores in detail the recently discovered synergistic effects between entomopathogenic fungi (EPFs), entomopathogenic nematodes (EPNs), and semiochemicals by developing innovative co-formulations, making use of strategies derived from nature. These co-formulations will be based on capsules containing EPFs (*Metarhizium brunneum or Beauveria bassiana*) in combination with strains of EPNs (*Heterorhabditis bacterio-phora*), or semiochemicals. Additionally, INBIOSOIL will develop integrated pest management (IPM) strategies that exploit synergies between these biocontrol agents and semiochemicals. The overall aim of the project INBISOIL is to optimize the use of biocontrol agents in the soil for more efficacious, low input, control of pests in farming systems of major importance in Europe. New crop protection strategies will be developed that will i) reduce pesticide inputs, i) provide protection in non-sterile soils, eliminating for soil sterilants, iii) reduce production costs, and iv) result in the production of high-quality and safer crops in accordance with theme priority area (Integrated pest management in farming systems of major importance in Europe).

Poster / Fungi. Wednesday, 16:30. **FU-36**

Oxidative stress levels in the entomopathogenic fungus *Beauveria bassiana* growing in very long-chain hydrocarbons

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Broad host range entomopathogenic fungi attack insect hosts via attachment to insect surface, with the subsequent production of degrading enzymes that help penetration through the cuticle.The outermost insect surface is covered by a lipid-rich layer, usually composed of very long-chain hydrocarbons. It is known that *B. bassiana* is able to grow on straight chain hydrocarbons (alkanes) as a sole source of carbon and energy, but it would have to pay a high cost to do so. The aim of this work was to study the oxidative stress levels in alkane-grown *B. bassiana*. For this purpose, we analyzed the gene expression pattern of *sod1*, *sod2*, and *sod3* encoding superoxide dismutases, *catA*, *catB*, *catC*, *catD*, and *catP* encoding catalases, and *gpx* encoding glutathione peroxidase; and the enzymatic activity of SOD, CAT, and GPx in crude homogenates. Fungi grown either in hexadecane (*n*-C16) or octacosane (*n*-C28) showed overlapping but differential gene induction, with a concomitant increment in enzymatic specific activities, compared with controls grown in complete medium. These results confirm that high levels of reactive oxygen species are produced in *B. bassiana* during growth in alkanes, and an antioxidant response is triggered in fungal cells to overcome this drawback.

MICROBIAL CONTROL

Poster / Microbial Control. Wednesday, 16:30. **MC-1-STU**

Fungal strain selection and screenhouse evaluation of the virulent isolate against aphids on crucifer and okra vegetables

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Aphids are major pest problems of crucifer and okra vegetables in sub-Saharan Africa. Biopesticides are now acceptable pest control alternatives to synthetic chemical insecticides. Five isolates of *Metarhizium anisopliae* and three of *Beauveria bassiana* were screened for virulence against the following apterous adult aphids in the laboratory: *Brevicoryne brassicae* and *Lipaphis pseudobrassicae* on kale, and *Aphis gossypii* on okra. *Metarhizium anisopliae* isolates ICIPE 30, ICIPE 62 and ICIPE 69 outperformed the others causing mortality of 85-98%, 83-97%, and 73-77%, in *B. brassicae, L. pseudobrassicae* and *A. gossypii*, respectively, at 5 d post inoculation. However, *M. anisopliae* ICIPE 62 had the shortest LT_{50} values of 2.8, 2.1 and 1.9 d; and the lowest LC_{50} values of 5.5×10^5, 8.1×10^4 and 1.7×10^4 conidia ml^{-1} against *A. gossypii, B. brassicae* and *L. pseudobrassicae*, respectively. It also produced significantly higher conidia on cadavers compared to the other isolates, and was therefore selected for screenhouse experiments. In the screenhouse, aqueous and oil formulations of ICIPE 62 significantly reduced aphid population growth rate (r_i), *B. brassicae* -0.03 and -0.03 and *L. psudobrassicae* -0.02 and -0.04 on kale, and *A. gossypii* -0.04 and -0.07 on okra, respectively; compared to the control (0.08 and 0.04 for *B. brassicae,* 0.01 and 0.01 for *L. pseudobrassicae,* and 0.03 and 0.01 for *A. gossypii*, respecively). These results are indicative of the potential of isolate ICIPE 62 in the management of aphids

Poster / Microbial Control. Wednesday, 16:30. **MC-2**

Virulence of fungal spores produced in liquid and solid state media on nymphs of *Trialeurodes vaporariorum*

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Quality of spores of five fungal entomopathogens, produced in liquid and on solid media, was assessed on nymphs of whitefly *Trialeurodes vaporariorum*. Isolates of *Lecanicillium attenuatum*, *L. muscarium*, *L. longisporum* and one unidentified *Isaria* sp. were first passed through larvae of *Tenebrio molitor* to enhance virulence. Three-times subcultured pure colonies were used to inoculate liquid or solid media to produce submerged and aerial spores. The liquid medium production system consisted of 250 mL Erlenmeyer flasks containing a mineral solution with a C/N ratio of 10/1 supplemented with yeast extract, placed in an orbital shaker at 180 rpm and 25°C. The solid medium production system consisted of Petri dishes containing PDA, placed in an incubator at 25°C. Spores were collected and suspensions of $1x10^6$ germinable spores were prepared. Five tomato leaves, infected with *T. vaporariorum* nymphs at 2^{nd}-3^{rd} instars, were submerged for one minute in the spore suspensions of each isolate, and maintained in 200 mL water-agar glasses in a growth chamber during ten days. The number of dead nymphs was evaluated six and ten days after inoculation. Control treatments consisted of ten leaves infected with the whitefly nymphs and treated with sterile water. Aerial spores of the *Lecanicillium* spp. isolates caused higher mortality than submerged spores. *L. longisporum* was the least affected by the production system. Contrary to *Lecanicillium*, submerged spores of the *Isaria* isolate killed more nymphs than aerial spores six days after inoculation. The production system should be considered during the screening and evaluation of microbial control agents.

Poster / Microbial Control. Wednesday, 16:30. **MC-3-STU**

Development of entomopathogenic fungi in mosquito control: which kind of production for which efficiency?

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Mosquitoes (Diptera: Culicidae) are zoonotic vectors responsible for numerous infectious diseases of medical and veterinary importance such as filariasis, malaria and encephalitis. As part of an integrated vector control, entomopathogenic fungi could be developed as biopesticides in two ways: spores and metabolites recognized as effective virulence factors. Solid-state fermentation enhances spore production and induces the secretion of metabolites quantitatively and qualitatively different from submerged fermentation, which impairs fungal metabolic efficiency. In this context, we showed high spore productivity of solid-state media based on agro-industrial substrates as wheat bran. Spores remained pathogenic, as revealed by classical toxicity tests and electron microscopy. However, the absence of free water makes culture parameter variations difficult to control in large-scale. Recently, we performed a bioreactor design intended for simultaneous spore and metabolite production, combining the technological advantages of submerged and solid-state fermentations.Biofilm fermentation (i.e. growth of fungal biomass on an inert support immerged in a nutrient medium) is a tremendous production system favouring the secretion of insecticidal metabolites in the liquid medium as we showed recently. This is also an interesting tool to provide an overview of the complexity of the metabolic pathways involved in the regulation of extracellular metabolites secretion because corresponding genes are reported to be differentially expressed from classical fermentation systems. Researches in vector control are currently intensified. In this context, the identification of genes and metabolites specifically expressed during biofilm fermentation will help to develop new technologies related both to the design of bioreactor and the production of insecticidal proteins.

Poster / Microbial Control. Wednesday, 16:30. **MC-4**

The basis for rootstock resilient to *Capnodis* species: screening for genes encoding delta-endotoxins from *Bacillus thuringiensis*

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Conventional methods often fail to control the flatheaded borers *Capnodis* spp, major pests of stony fruit trees; the larvae are protected from insecticides and predation because they feed deep in the roots. A potential solution is transgenic trees producing in their roots toxic compounds such as Cry proteins of *Bacillus thuringiensis* (*Bt*). Toxicities against *Capnodis* larvae were demonstrated by exploiting a recently-designed artificial larval diet and an available collection of field isolated *Bt*. An isolate of *Bt tenebrionis* (*Btt*) from commercial bioinsecticide (Novodor) displayed LC_{50} and LC_{95} values of 3.2 and 164 mg g^{-1} respectively against neonates of *Capnodis tenebrionis*, whereas values of the most toxic field isolate K-7 were 1.9 and 25.6 mg g^{-1} respectively. Weights of surviving larvae after 1 month on diets containing low concentrations of K-7 (0.1 - 1.0 mg g^{-1}) were lower than on *Btt* or untreated larvae. K-7 was also toxic against larvae of *C. cariosa* and *C. miliaris* and found to harbor genes encoding Cry9Ea-like and Cry23Aa/Cry37Aa binary toxins. Larvae of *Capnodis* spp. are susceptible to *Bt* Cry toxins. Expressing *cry* genes active against these pests thus seems a feasible solution toward production of transgenic rootstock trees resilient to the pest

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Poster / Microbial Control. Wednesday, 16:30. **MC-5**

Selection of entomopathogenic fungi for the control of *Aegorhynus nodipennis* (Coleoptera: Curculionidae) under laboratory conditions

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Plum weevil *Aegorhynus nodipennis* is one of the most important native pests of blueberry in Chile. The larvae produce severe damage to the root system, by destroying the plant crown, and causing decay and death of the plant within a few years. Adults are long-lived and feed on twigs during the day and oviposit on the crown of the plants, where they hide during the night. Strains of the insect pathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* were evaluated on adults of the Plum weevil under laboratory conditions. 45 strains of *M. anisopliae* and 50 strains of *B. bassiana,* from the Chilean collection of insect pathogenic fungi where screened. Plum weevil adults were exposed to a dose of 1×10^7 conidia / insect and mortality was assessed every day for up to 10 days. Two strains of *B. bassiana* and *M. anisopliae* were selected as the most effective on adults. The *B. bassiana* strain reached 100% mortality and the *M. anisopliae* was only 80% of control. Attributes such as high performance of the spore, stability and virulence will determine the selection of strains to be evaluated in greenhouse and field trials.

Poster / Microbial Control. Wednesday, 16:30. **MC-6**

Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* strain HD1

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The development of insect resistance to *Bacillus thuringiensis* (Bt) appears to involve various mechanisms and to be dependent on the type of insect, toxin, and Bt strain. The aim of this research was to investigate the factors affecting the susceptibility of insects to Bt (protein level, the midgut bacteria, and mutations in the *ABCC2* gene), in five Brazilian populations (PC, PA, PX, SBT and BT) and one English population of *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae). The BT population of *P. xylostella* showed a high resistance to the HD1 strain, and therefore was used in the molecular assays. Enzymatic and molecular experiments with the guts of larval populations were also conducted to investigate the factors affecting the susceptibility of insects to

Bt. We analyzed total protein, total protease, protease activity, esterase levels, intestinal bacteria, and exon characteristics. Mutations in the *ABBC2* gene may be related to resistance to Bt in various insects, as deletion of this gene occurs in a resistant strain of *P. xylostella*. The exon that has a known mutation in the (NO-QAGE) Bt-resistant population was sequenced. None of the populations showed this or any other mutations in the exon. Gut bacteria may influence the susceptibility of insects to Bt and all sequences had similarities above 99% for the *Enterococcus mundtii* 16S rRNA gene. The tests performed, both enzymatic and molecular, were inconclusive as to the factors that may influence the susceptibility of *P. xylostella* to Bt and further studies should be conducted to elucidate these factors.

Poster / Microbial Control. Wednesday, 16:30. **MC-7**

Sublethal effects of the Cry1Ac toxin of *Bacillus thuringiensis* Berliner in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations

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The diamondback moth (DBM), *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is a key pest of crucifers. Although can be controlled with insecticides, *P. xylostella* can quickly develop resistance to insecticides, such as those of from *Bacillus thuringiensis*. The objective of this research was to analyze the sublethal effects of *B. thuringiensis* Cry1Ac protein in five Brazilian populations of *P. xylostella* (PC, PA, PX, SBT, and BT. Bioassays examining the sublethal effects of Cry1Ac protein in DBM larvae were conducted using concentrations of 0.1, 0.25, and 0.5 µg/mL for the BT population, and 0.001, 0.005, 0.01, and 0.05 µg/mL for the PA, PX, PC and SBT populations. As a control treatment, autoclaved deionized water and 50 µg/mL Triton-X100® was used. The period of life from the third instar to pupa, pupal period, pupal weight, sex ratio, survival of from the third instar to pupal stage, survival of from the third instar to adulthood, and leaf consumption by the larvae were all evaluated for sublethal effects. Sublethal effects on the Bt population were most significant in prolonging the larval period, for approximately 2 days with a toxin concentration of 0.05 µg/mL, and the emergence of adults was 44% lower than that in the control. For the PA, PC, SBT, and PX populations, the most significant sublethal effects observed were also in prolonging the larval period and adult emergence. No influence on consumption of the larvae was observed, except with the *Bt* population, where the consumption was significantly lower at all tested concentrations.

Poster / Microbial Control. Wednesday, 16:30. **MC-8**

Effect of *Bacillus thuringiensis* Berliner on biological characteristics of *Orius insidiosus* Say (Hemiptera: Anthocoridae) fed with eggs of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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The Diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is considered the most important pest of Brassicaceae (Cruciferae) worldwide, occurring throughout the year in Brazil, where chemical control is the most widely used method, justificated by the convenience, quick action and efficiency. The indiscriminate use of pesticides can affect non-target organisms, and the growing concern for the environment, the high cost of pesticides and frequent cases of resistance in populations of increased interest in the use of other control tactics as entomopathogenic organisms such as *Bacillus thuringiensis* and predators such as *Orius insidiosus*. The objective of this work is to evaluate the action of *B. thuringiensis* (Agree ®) in biological characteristics of *O. insidiosus*. The predators were fed with eggs of *P. xylostella* treated with distilled water (control) and a suspension Agree® (*B. thuringiensis aizawai* CG91), at a dosage of 0.7 g/0.5L. The nymphal period, consumption and nymphal survival rate were assessed, whereas with adults were measured consumption, the number of eggs per female and egg viability Parameters were also determined for the construction of fertility life tables for eggs treated and not treated with *B. thuringiensis*. The parameters duration of the second instar, nymph consumption and female longevity of *O. insidiosus* are affected by the presence of Agree ®, and females who consume eggs treated have the progeny decreased, resulting in lower population growth rate.

Poster / Microbial Control. Wednesday, 16:30. **MC-9-STU**

Evaluating microbial biocontrol agents: effects of *Metarhizium brunneum* on a non-target arthropod

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The objective of this study was to evaluate the virulence of *Metarhizium brunneum* on non-target arthropods. The strain Met52/BIPESCO 5/F52 is used against various pest insects throughout Europe and North America. However, research related to the ecotoxicology and side effects against non-target organisms are still needed. In this MSc study, a part of the EU supported project INBIOSOIL, we documented that *M. brunneum* had a high virulence against the model insect *Tenebrio molitor* (Coleoptera: Tenebrionidae), whereas it had a much lower virulence against the beneficial arthropod *Atheta coriaria* (Coleoptera: Tachnynidae), a soil dwelling predator used for macrobiological control. In addition, the virulence of *M. brunneum* was compared to that of another entomopathogenic fungus *(Beauveria bassiana*). Bioassay results showed notable efficacy of the entomopathogenic fungi against *T. molitor*, both at high and low spore concentrations (respectively 1×10^7 and 1×10^5 conidia/ml). Conversely, infection bioassays carried out on *A. coriaria* showed significantly lower virulence of the fungal isolates at a high spore concentration. These data suggest that this *M. brunneum* strain does not represent a threat to the non-target arthropod *A. coriaria*. Further studies are still needed to evaluate the effects of *M. brunneum* on other non-target arthropods. Nevertheless, based on the results of this study we propose that *M. brunneum* can be considered a 'low risk substance', a novel category of plant protection agents currently considered by the EU Commission.

Poster / Microbial Control. Wednesday, 16:30 **MC-10-STU**

An experimental autoinoculation device to control an invasive Asiatic pest, Drosophila suzukii

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Unlike most drosophilids, which typically infest overripe or decaying fruit, it has been observed that Drosophila suzukii (Matsumura) also oviposits eggs into the skin of immature and/or ripening fruit through the use of a serrated ovipositor. Drosophila suzukii is an important pest of fruit such as strawberry, cherry, blackberry, blueberry, peach, plum, nectarines and grapes. Spotted wing D. suzukii was first found in Spain in 2008. Managing this pest is a challenge, and new methods of control are being developed. In our research, the transmission potential of EAMa 01/58-Su Metarhizium brunneum strain was evaluated against D. suzukii adults in experiment cages, using an experimental autoinoculation device which consists in a plastic mineral water bottle with fermented food as lure, and a tissue with the fungal propagules. D. suzukii adults entered and exited the autoinoculation device for the 48 h of exposure and became infected with the fungus with 100.0% mortality followed by mycosis. These results show the potential of the lure and infect as a strategic option for the control of D. suzukii using EAMa 01/58-Su strain, with the persistence of the inoculum in the device and the time course evolution of the adult fly infection being actually investigated.

Poster / Microbial Control. Wednesday, 16:30 **MC-11**

Use of a commercial *Metarhizium anisopliae* s.l. formulation to control *Rhipicephalus microplus* ticks in pen study

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The present study evaluated the effect of the commercial product Metarril® SP Organic of *Metarhizium anisopliae* s.l. plus 10% mineral oil to control *Rhipicephalus microplus* ticks in a pen study. Three groups were formed with six animals each: the first group was exposed to Metarril® plus 10% mineral oil; the second group was exposed to sterile distilled water plus 10% mineral oil (oil control group) and the third group received no treatment (control group). Fungal formulation contained 1 x 10^8 conidia mL^{-1}. Each animal was sprinkled with 3L of formulation. Fallen ticks were counted daily and a sample of 20 engorged females per group was incubated for assessment of biological parameters. Throughout the study period, Metarril® oil-based formulation showed an efficiency ranging from 19.20% to 67.39% in comparison with the control group; and from 8.18% to 61.38% in comparison with the oil control group. Average efficiency of Metarril® oil-based formulation was 47.74% and 40.89% in comparison with control and oil control groups, respectively. Changes in the biological parameters of *R. microplus* females were observed in the first three days after treatment. There was statistical significant reduction in females' egg mass weight, larval hatching percent, nutritional index and egg production index. We concluded that Metarril® SP Organic plus 10% mineral oil was efficient against *R. microplus* ticks in pen studies. Further *in vivo* studies are required in order to increase efficiency of this product aiming establish a protocol for the use of Metarril® in field conditions against the cattle tick.

Poster / Microbial Control. Wednesday, 16:30. **MC-12**

Two Colombian entomopathogenic fungi are highly efficient on *Cerotoma tingomariana*

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The Chrysomelids (Coleoptera: Chrysomelidae) are a limiting soybean pest in Colombia. These insects can affect seeds, nodules, leaves and roots, reducing the yield crop. An amount of 19 species has been registered, but *Cerotoma tingomariana* is the most important, due to its high frequency and distribution. This insect is controled with insecticides (I - II category) and some of them are forbid in USA or Europe. The aim of this work was to select an efficient entomopathogenic fungus on *C. tingomariana.* Seven isolates of *Beauveria bassiana* (Bv) and six isolates of *Metarhizium anisopliae* (Mt) were biological testing on laboratory. In addition, this isolates were tested on different temperatures (5ºC, 15ºC, 25ºC, 30ºC and 35ºC), pH values (3, 5, 7, 9) and tolerance to UVB radiation (302 nm) by measuring germination (%), radial growth and Colony Formate Unit (CFU). Mt isolates showed efficiency under 50%. Isolates Bv060 and Bv003 showed an efficiency of 100%. In the UVB radiation test, Bv060 reduced the conidia viability between 75% and 80%, and Bv003 reduced the viability between 65% and 66%. At 5 and 9 pH value, the two isolates (Bv003 and Bv060) showed germination higher than 90% and the faster rate of radial growth. Bv003 showed the best growth at 15ºC and 25ºC and Bv060 at 25ºC and 30ºC. These results suggested that Bv060 and Bv003 could be use as an active principle for a biopesticide on *C. tingomariana* control in soybean.

Poster / Microbial Control. Wednesday, 16:30. **MC-13-STU**

Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana*

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Pollen beetles are a main pest in oilseed rape (OSR) throughout Europe, able to cause substantial yield loss. The main damage is caused by adult beetles feeding on pollen in spring during bud stage of inflorescences. There is currently no possibility to control pollen beetles in organic OSR cultivation. In addition, increasing resistance of pollen beetles to commonly used insecticides hampers conventional OSR production and further emphasizes the need for alternative control possibilities.
The application of entomopathogenic fungi (EPF) is a promising tool in biological control of pollen beetles (Hokkanen 2008). Several Swiss isolates of the EPF *Beauveria bassiana* showed promising effects in laboratory experiments, causing up to 80% mortality seven days after application (Kuske 2011). Field treatments showed similar results regarding beetle mortality, but did not result in significantly increased yield so far. To improve their efficacy, synergies of EPF and

other natural compounds, such as stone dusts or vegetable oils, are tested. First laboratory results of combined applications of *Beauveria bassiana* spores and vegetable oil have shown a potential increase in beetle mortality due to improved fungal infection. The exploitation of synergistic effects and innovations in formulation technology should result in a better spore persistence under field conditions and a higher efficacy of the fungal treatments against pollen beetles.

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Poster / Microbial Control. Wednesday, 16:30. **MC-14**

Pathogenicity and virulence of *Beauveria* spp. against mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytidae)

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The mountain pine beetle (MPB), is a forest pest to western Canada and the United States and causes severe disturbance in lodgepole and other pine forests. We evaluate pathogenicity and virulence of number of *Beauveria* spp. including the two commercial strains of *B. bassiana*, GHA and Naturalis against adult MPB. All the 29 isolates tested in the preliminary bioassay proved to be pathogenic to MPB adults. Mean survival times (MST) of MPB adults when treated with 1 $\times 10^6$ conidia/ml falls between 4.05 to 8.95 days and the commercial isolate GHA is the most virulent (MST 4.05 d), followed by isolates INRS 211(MST 4.59 d), and INRS 236 (MST 4.82 d) based on the log rank test. Among the 3 different species tested, *B. bassiana* isolates were highly virulent followed by *B. pseudobassiana*. The *B. brongniartii* isolates used in this study were neither virulent nor supported conidia growth on the cadavers. From this initial screening, seven isolates of *B. bassiana* viz., GHA, Naturalis, INRS 211, INRS 236, INRS CFL-A, L49-1AA, and ARSEF 8150, were selected based on their virulence as well as mycosis/condiosis for further dose-wise bioassay. Based on the LC_{50} values, the commercial isolates, GHA and Naturalis were the most virulent to MPB, however, isolates INRS 236 and INRS CFL-A were the better conidia producer. The result obtained from this study was used in selecting amendable and virulent *Beauveria* isolates to be deployed in managing MPB through classical biological approaches in a trap based auto-contamination-dissemination strategy.

Poster / Microbial Control. Wednesday, 16:30. **MC-15**

The Use of Microbial Plant Protection Agents for Insect Control in Germany

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Micro-organisms play an important role in biological plant protection in Germany. By the Directive 2009/128/EC on the sustainable use of pesticides, biological control measures are proposed to be enforced in order to reduce the application of chemical pesticides in Europe. To obtain information about the scale of application of biological control agents in Germany, we have performed a survey on their use. Two baculoviruses are registered for tortricid control in Germany. The most important one is the *Cydia pomonella* granulovirus, which is used on about 30% of apple plantations in Germany. Three *Bacillus thuringiensis* (Bt) subspecies (kurstaki, azawai and tenebrionis) are in use and play an important role in organic farming and integrated pest management (IPM). So far, there is no entomofungal product registered as plant protection agent in Germany. However, some strains of *Beauveria bassiana*, *B. brongniartii* or *Metarhizium anisopliae* have been used for research purposes or for restricted use with a specific legal allowance. The data are presented in the Status Report Biological Plant Protection, which is published every five years by the Julius Kühn Institute and represents an indicator of the National Action Plan to monitor the use of plant protection products.

Poster / Microbial Control. Wednesday, 16:30. **MC-16-STU**

Synthesis and secretion of volatile organic compounds by *Triatoma infestans* infected with *Beauveria bassiana*

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Physically disturbed *Triatoma infestans* adults secrete volatile organic compounds (VOC) with alarm and defense function. It is still unclear whether infection with entomopathogenic fungi changes or not the profile of these volatiles. The aim of the present research was to study the effect of *B. bassiana* on secretion of VOC by *T. infestans* and to study the expression of genes potentially involved in the biosynthesis of these volatiles in triatomines infected or not. Volatiles released by *T. infestans* on different periods after treatment (1-4, 6-10, 11-15 days) were quantified and identified employing capillary gas chromatography coupled to mass spectrometry. The expression pattern of *Ti-brnq* and *Ti-bckdc* was analysed by real-time PCR, 4 and 10 days after treatment. Isobutyric acid was the most abundant VOC found (70 to 78% of the total) with no significant effect of the progress of infection on quantitative secretion of this compound. Secretion of propionic acid, however, was highest in the beginning (18.6±5.8%) and decreased distinctly with the progress of infection and at this time did not differ from values found for the control. Highest expression of both genes was found on insects 4 days after treatment. Significant difference was found in *Ti- brnq* expression, with 1.3±0.5 and 3.0±0.4 fold induction over the controls in insects treated with 1×10^6 and 1×10^8 con/ml, respectively. Similar results were observed for *Ti-bckdc* expression, resulting in 1.9±0.3 and 2.5±0.4 fold induction, respectively. The results help to understand better the impact of fungal infection on the chemical ecology of *T. infestans.*

Poster / Microbial Control. Wednesday, 16:30. **MC-17**

Preliminary studies of entomopathogenic microorganisms present in Latvian population of horse-chestnut leaf miner *Cameraria ohridella*

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The first record of horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae) in Latvia was made in summer 2002. In recent years *C. ohridella* has spread across all territory of Latvia. The aim of the study was to acquire preliminary data on mortality factors of horse-chestnut leaf miner *C. ohridella* and identify present entomopathogenic fungi and bacteria. Since 2010 *C. ohridella* population dynamics are monitored in two sampling plots. This work provides information about causes of mortality of *C. ohridella* larvae and pupae and gives first record about bacterial and fungal microflora of collected larvae. Observed larval mortality, caused by pathogens was low (0.2-1.6%). Specimens with symptoms of infection were used for pathogen isolation. Twelve species of entomopathogenic fungi were isolated from collected dead specimens. A pilot experiment to test virulence of fungal isolates *Beauveria bassiana*; *Isaria fomosoroseus* and *Metharizium anisopliae* on *C. ohridella* larvae and hibernating pupae was performed. Bacteria were isolated from insects by using standart methodology - dissecting insect and preparing homogenates. Individual bacterial isolates 16S rRNA genes were amplified and sequenced. Results showed that bacterial community is relatively simple and it's similar to composition found in other insect species described by the same methodology. Community was dominated by proteobacteria – *Pseudomonas* sp. and *Pantoea* sp.

Poster / Microbial Control. Wednesday, 16:30. **MC-18**

Toxicity of *Bacillus thuringiensis* BERLINER Cry toxins in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations

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Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), the diamondback moth (DBM), is a major insect pest of crucifers (Brassicaceae) worldwide. The most common insecticides used to control *P. xylostella* are based on the entomopathogenic bacterium *Bacillus thuringiensis* (Bacillaceae) (Bt). Although many studies have focused on the action of Bt on various agricultural pests, such as DBM, many doubts still persist, particularly regarding the toxicity of Bt proteins. We analyzed the virulence of Cry proteins in Brazilian populations of *P. xylostella*. Bioassays of susceptibility in five Brazilian populations (PC, PA, PX, SBT, and BT) of *P. xylostella* and Cry1Ac, Cry2Aa, and Cry1IE *B. thuringiensis* proteins, estimating the virulence of the toxins, were performed. Seven concentrations, ranging from 0.001 to 1.0 µg/mL, for the PA, PC, PX, and SBT populations, and 0.1 to 2.5 µg/mL for the BT population, were used to calculate the values of LC_{50}. Five replicates were performed, with each replicate being a petri dish containing 20 larvae, totaling 100 insects per concentration for each population. The Cry2Aa and Cry1IE toxins caused no mortality in larvae from any of the populations; therefore, tests were performed only with Cry1Ac. The PC, PA, PX, SBT, and BT *P. xylostella* populations exhibited different levels of susceptibility to the Cry1Ac toxin. The PA, PC, and SBT populations showed LC_{50} values of 0.02, 0.04, and 0.04 µg/mL. The LC_{50} estimate for the BT population was 0.78 µg/mL, while that for PX it was 0.01 µg/mL. The LC_{50} estimated for the BT population was 78 fold greater than that for the PX population.

Poster / Microbial Control. Wednesday, 16:30. **MC-19**

Bacillus thuringiensis isolation from Brazilian soil samples: molecular characterization and biological activity against _Plutella xylostella_ (Lepidoptera: Plutellidae)

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Plutella xylostella L. (Lepidoptera: Plutellidae) is one the most important pests of crucifer worldwide, and farmers usually control this pest with pesticides that favors the population resistance development, depletion of natural enemies and environmental pollution. Studies on biological control agents such as the entomopatogenic bacterium *Bacillus thuringiensis* must be carried out aiming to minimize or even replace the pesticides in the field. This research was carried out to isolate *B. thuringiensis* from 40 soil samples, to characterize them by Polymerase Chain Reaction (PCR) and mortality bioassays were performed to verify the *B. thuringiensis* biological activity of each isolate against 100 *P. xylostella* second instar larvae. 50 *B. thuringiensis* isolates were obtained from soil samples. No isolate amplified genes *cry1Ab, cry1Ac, cry1Ea, cry1Eb, cry1Fa, cry1Fb, cry2Aa, cry2Ab, cry2Ac, cry9A, vip1, cyt2B* and *cyt2Ba* but isolates named LCMA04, LCMA05 and LCMA29 amplified gene *vip2* and the isolates LCMA06, LCMA20, LCMA45 and LCMA46 amplified gene *cry1C* and *vip2.* These isolates were pathogenic to *P. xylostella* second instar larvae but the mortality range from 38,0% to 55,5%. This mortality is too low to consider these isolates as promising ones to *P. xylostella* management. This isolation is ongoing to find isolates with high virulence to *P. xylostella*.

Poster / Microbial Control. Wednesday, 16:30. **MC-20-STU**

Effect of endophytic *Beauveria bassiana* on herbivore defence in *Arabidopsis thaliana*

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The entomopathogenic fungus *Beauveria bassiana* can live as an endophyte by colonizing plant tissues without causing disease symptoms. Recent studies in different crop plants indicated that endophyte presence can have a negative effect on herbivorous insects. However, whether this was due to induced plant defence responses has not been reported. We established *Arabidopsis thaliana* as a model plant to find out whether *B. bassiana* colonization increases herbivore resistance by activating/priming the jasmonic acid (JA) or salicylic acid (SA) defence pathways. Three *B. bassiana* strains were applied as conidial suspension to *Arabidopsis* using root dipping. Colonization was assessed through plating on selective medium and through PCR based detection using *B. bassiana* specific SCAR markers. The endophyte was recovered from leaves and inflorescence confirming systemic colonization throughout the plant. Bioassays were carried out to test the effect of endophyte presence on caterpillars of *Plutella xylostella* and the aphid *Myzus persicae.* Endophyte presence did not have any antagonistic effects on the growth of *P. xylostella* and the fecundity of *M. persicae*. The re-

isolated fungus caused 100% mortality when applied topically on caterpillars. This correlated with the finding that JA levels were only induced by caterpillar feeding but were not influenced by the presence of the fungus. No effect by either treatment was found on endogenous SA levels. In conclusion, our results do not confirm that endophytic *B. bassiana* induces plant defences against the selected herbivore species. Further studies are planned to assess the plant's transcriptomic response to the presence of this endophytic entomopathogen.

Poster / Microbial Control. Wednesday, 16:30. **MC-21-STU**

Pathogenicity of *Beauveria* and *Metarhizium* to the two stink bug species *Nezara viridula* and *Piezodorus guildinii* (Hemiptera: Pentatomidae) in laboratory and semi-field

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The stink bug species *Nezara viridula* and *Piezodorus guildinii* are troublesome pests of common bean (*Phaseolus vulgaris*)**.** The aim of this study was therefore to determine the pathogenicity of two *Beauveria* isolates (Bb-1 and Bb-18) and two *Metarhizium* isolates (Ma-11 and Ma-30) form Cuba to these two stink bug species. Each fungal strain was tested in the laboratory against adults of the two stink bug species. Further, in a pilot semi-field experiment the two stink bug species inoculated on bean plants with pulses in cages were sprayed with the same four fungal strains In the laboratory experiment Ma-30 and Ma-11 caused 100% mortality in both stink bug species. The *Beauveria* strains resulted in a lower mortality, however, and Bb-1 caused 85% mortality in both stink bug species, while Bb-18 caused 85 % mortality in *P. guilninii* and 95% mortality in *N. viridula*. In the semi-field experiment the Ma-30 strain caused the highest mortality and 73% of the *N. viridula* was killed by this fungus while only 68% of the *P. guildinii* was killed. The Ma-11 strain caused 65 % mortality in *N. viridula* and *P.guildinii* while Bb-18 and Bb-1 caused 41% and 54% mortality respectively in both stink bug species.

Poster / Microbial Control. Wednesday, 16:30. **MC-22-STU**

Evidence for synergies between *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae) and *Metarhizium brunneum* (Hypocreales: Clavicipitaceae) in western corn rootworm control

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The western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelida), is one of the most deleterious pests of maize worldwide, and is commonly controlled by chemical insecticides. Currently neonicotinoid-dressed maize seeds are banned in the European Union which highlights the importance of intensified research into suitable alternative control strategies. Field trials using a blend of entomopathogens in conjunction with chemical insecticides were carried out to determine the effect on survival and development of the WCR as well as on grain yield. The entomopathogens included the nematode species *Heterorhabditis bacteriophora* Poinar (Heterorhabditidae) and the fungus *Metarhizium brunneum* Petch (Clavicipitaceae). The agents were applied in two naturally heavily WCR-infested maize fields in the province of Styria, Austria, in 2013. Neither the abundance of larvae nor the number of adults showed significant differences between the treatments. However, when both *H. Bacteriophora* and *M. brunneum* were used in combination with untreated seeds, the grain yield was almost equivalently high compared to treatments using neonicotinoid-dressed seeds. The two entomopathogens possibly interact synergistically and could provide a powerful alternative strategy to chemical insecticides for the larval control of *D. v. virgifera*. Nonetheless, a repetition and extension of the trials in 2014 is essential to further evaluate the efficacy of the different agents for WCR control.

Poster / Microbial Control. Wednesday, 16:30. **MC-23**

Evaluation of the effectiveness of the entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat

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Wireworms, the larval stage of Elaterid beetles are serious soil dwelling pests of small grain, corn, sugar beet and potato crops. *Limonius californicus* (Mannerheim) and *Hypnoidus bicolor* (Eschscholtz) are the predominant wireworm species infesting wheat in Montana, particularly in north-central Montana. Currently available insecticides provide only partial control, and no alternative management tools exist. At two field locations (Ledger and Conrad, MT) in 2013, the fungi, *Metarhizium brunneum* F52, *Beauveria bassiana* GHA, and *Metarhizium robertsii* DWR 346, were evaluated in seed coat, in-furrow granular and soil drench applications, in addition to imidacloprid seed treatment, which is currently being used by growers. Wireworm damage in various treatments was evaluated as standing plant counts, wireworm population survey, and grain yield production. The three fungi applied as formulated granules or as soil drenches, resulted in significantly higher plant stand counts and yields at both locations, than fungus-coated seed treatments and the untreated control. Significant difference was detected among the application methods instead of species of the fungi. All three fungi applied as granules in furrow and in soil drench were paramount to seed-coating treatments in wireworm control, and provided an efficacy comparable or superior to imidacloprid. The fungi used in the current study provided significant plant and yield protection under moderate wireworm pressure, indicating their potential utility in the integrate management of this pest.

Poster / Microbial Control. Wednesday, 16:30. **MC-24-STU**

Using the combination of entomopathogenic fungi and extracts improves control of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

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Both the virulence and the insecticidal activity of the crude extracts of 26 isolates of the mitosporic ascomycete entomopathogenic fungi *Metarhizium* sp. and *Beauveria* sp. (Ascomycota, Hypocreales) were determined against the second-instar *S. littoralis* larvae (Boisduval) (Lepidoptera, Noctuidae), which isconsidered a very harmful polyphagous insect pest. All isolates were pathogenic for second instar *S. littoralis* larvae by immersion on fungal suspension, but only four isolates of *Beauveria* (EABb 01/33-Su, EABb 01/88-Su, EABb 01/103-Su, and 3155) and one isolate of *Metarhizium* caused more than 50% mortality of larvae. EABb 01/33-Su and EABb 01/88-Su isolates caused the higher mortalities with 78.33% and 75.00%, respectively, and their average survival time (AST) values were 9.67 and 8.73 days, respectively. The LD_{50} and LT_{50} values were $5.69x10^6$ conidia ml^{-1} and 6.76 days for EABb 01/33-Su and $1.05x10^7$ conidia ml^{-1} and 7.02 days for EABb 01/88-Su. On the other hand, the crude extracts obtained from the isolates EAMb 09/01-Su and EAMa 01/58-Su caused the highest mortality rates, 80.00 and 66.66%, and the lowest AST values, 5.13 and 4.43 days, respectively. Topical application of the crude extracts did not cause any mortality. Combined treatments of fungal suspensions of isolates EAMb 09/01-Su and EAMa 01/58-Su and their extracts caused higher mortality rates than the single ones, in a dose-dependent manner, with mortality rates reaching 100% for EAMb 09/01-Su isolate and its extract at 1 mg ml^{-1} and 76% mortality for EAMa 01/58-Su, and its extract at 1 mg ml^{-1}.The combination of the fungus EAMb 09/01-Su at 10^8 conidia.ml^{-1} and the crude extracts had a synergistic effect on larvae resulting in 100 % mortality to concentrations 1 mg protein ml^{-1} and the combination of the fungus EAMb 01/33-Su + extracts EAMb 09/01-Su to concentration of 10^7 and 10^8 conidia ml^{-1} to 1 mg protein ml^{-1}crude extract also had a synergistic effect on larvae resulting in 93.33 and 100% mortality. The AST ranged between 4.08 and 5.77 days at 10^8 conidia.ml^{-1}. These results show the potential of using the combination of entomopathogenic fungi with crude extracts for an integrated *S. littoralis* management strategy targeting larvae.

Poster / Microbial Control. Wednesday, 16:30. **MC-25-STU**

Wireworm control with fungus colonized barley kernels in cover-crops

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Insecticide treatments to control wireworms in cover-crops have been a successful strategy to prevent wireworm damage in sensitive crops planted in the following season. One example was the application of Fipronil as a seed treatment of summer oat preceding potatoes. This way, the wireworm population was reduced below the damage threshold already before planting of potatoes.
We tested a similar strategy in a semi-field pot experiment, replacing the insecticide with *Metarhizium brunneum* ART2825, formulated as fungus colonized barley kernels (FCBKs). Pots were treated with four different doses of FCBKs in August 2013 during sowing of summer oat. In addition, pots were artificially infested with *Agriotes obscurus* larvae. In April 2014, potatoes were planted into these pots. Establishment of the fungus in the pots was evaluated by counting colony forming units per g of substrate. Numbers of recaptured wireworms and the percentages of wireworms dying from mycosis were used to estimate efficacy of the treatments. Finally, effect on yield will be as estimated by counting wireworm holes on harvested potatoes.
Preliminary results are promising: The fungus successfully established in the substrate after a few weeks and up to 70% of wireworms were killed by the treatments, depending on FCBK doses used for application. Results suggest that treating cover-crops with *Metarhizium*-inoculated FCBKs may be a useful tool for biological control of wireworms in potatoes.

Poster / Microbial Control. Wednesday, 16:30. **MC-26**

A resource efficient method to test non target effects of new biocontrol agents in vitro

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As part of the EU supported project INBIOSOIL we developed a protocol to test the non-target effects of microbial biocontrol agents and their formulations. For this purpose we selected four beneficial - predatory arthropods that are widespread and naturally occurring in Europe and are all also commercially available for biological pest control: *Aphidoletes aphidimyza* (Insecta, Diptera, Cecidomydiidae), *Atheta coriaria* (Insecta, Coleoptera, Staphylinidae), *Orius majusculus* (Insecta, Hemiptera, Anthocoridae) and *Geolaelaps aculeifer* (Acari, Mesostigmata, Laelapidae). These arthropods have different life cycles, prey, and most important, they inhabit different strata of the plant and soil in the field. The protocol allows a quick assessment of the potential side effects of microbiological biocontrol agents and their formulation components on these representatives of beneficial arthropods – and therefore should be considered standard tests to be done before further resource and time demanding testing in the field.

Poster / Microbial Control. Wednesday, 16:30. **MC-27**

Ultrastructure of midgut of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) after consumption of prey with the *Bacillus thuringiensis* strain HD1

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The interaction of Cry toxins from *Bacillus thuringiensis* in the midgut of some insect larvae determines their efficacies as insecticides, due to the expression and availability of the sites of action of the toxins in the midgut. Research has highlighted cases of resistance to Cry toxins due to alterations in the binding sites in columnar cell membranes. We analyzed the effects of spraying a *B. thuringiensis* var. *kurstaki* (HD1 Strain) suspension at a concentration 3×10^8 spores/mL, onto leaves that were then offered to the larvae of *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) and subsequently offered as prey to the predator *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae). We examined the ultrastructure of the midgut of predators. *P. nigrispinus* adults, 3 h after consuming prey with the HD1 strain were used for dissection and extraction of the midgut. The villi present in the midgut of the predator were observed in both cross section and as longitudinal sections. At the apex of the intestinal cells, the microvilli were seen. Also visible were remarkable muscle fibers in the lumen of the intestine; these fibers are perceptible only in the anterior and middle intestine, suggesting that they move when moving food into the large intestine during

digestion. The results showed that there were no adverse effects on the predator when the larvae of *P. xylostella* had previously ingested the HD1 strain of *B. thuringiensis*.

Poster / Microbial Control. Wednesday, 16:30. **MC-28**

Control of sugarcane borer, *Diatraea saccharalis*, with formulations of *Beauveria bassiana* and *Metarhizium anisopliae*

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The control of sugarcane borer (*Diatraea saccharalis*), the most important pest of this crop, with entomopathogenic fungi has already been reported in Brazil. However, have been used the pure conidia, which can decrease the efficiency of control due to environmental factors such as temperature and level of ultraviolet radiation. The objective of this study was to evaluate, in laboratory, encapsulated formulations containing *Beauveria bassiana* and *Metarhizium anisopliae*, against this pest. It was used pure conidia of the isolates IBCB 66 (*B. bassiana*) and IBCB 425 (*M. anisopliae*) and the formulation in sodium alginate. The fungi, were applied in two ways, powdered and sprayed, at the concentration 6 x 10^8 conidia, and the formulation was applied directly in two concentrations 6 x 10^8 and 1 x 10^9. The caterpillars were evaluated at the 7^o and 14^o day after the application. The jars with insects were kept in air-conditioned room at 25.0 ^oC ± 2,0 ^oC and relative humidity around 70%. The bioassay was done with 30 caterpillars per treatment and 5 repetitions. To pure conidia of *B.bassiana*, in the 14^o day, the mortality of caterpillars was 96% in sprayed application, while in powdered 87%. In the formulation, the mortality was 57% at the concentration of 6 x 10^8 and 77% at 1 x 10^9. As for the *M. anisopliae*, the mortality of caterpillars in the 14^o day, in the sprayed treatment was 47%, and in the powdered 27%, while the mortality in the formulations were 4% at the concentration of 6 x 10^8 and 24% at a concentration of 1 x 10^9.

Financial support: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo).

Poster / Microbial Control. Wednesday, 16:30. **MC-29-STU**

Identification and functional analysis of two ABCC family genes in *Helicoverpa armigera*

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Bt toxins are widely used for insect control and resistance to Bt toxin is a problem that has been presented in recent years. Midgut receptors have been reported as binding proteins for Bt toxins and play important roles in toxicity. Recently, mutations in the ABCC2 transporter were reported to take key roles in *Bt* resistance of several species of insects. In this study, we cloned two ABCC genes from *Helicoverpa armigera,* and sequence analysis showed that these genes were quite homologous to ABCC2 and ABCC3 genes from other lepidopteran insects, so were named HaABCC2 and HaABCC3 respectively. Tissue specific expression and instar specific expression analysis showed that the two ABCC genes were mainly expressed in midgut and later instar larvae. RNAi was done to silence these ABCC genes by feeding dsRNA to *H. armigera.* Bioassays showed that silencing of *HaABCC2* in *H. armigera* larvae resulted in increased survival and pupation rates with normal eclosion rate on Cry1Ac toxin-incorporation diet, while silencing of *HaABCC3* had no effect. Our research proved that *ABCC2* play important role in Cry1Ac toxin pathological mechanism in *H. armigera*.

MICROSPORIDIA

Poster / Microsporidia. Wednesday, 16:30. **MI-1**

Decline of native bumblebees (*Bombus*) and *Nosema* (Microspordia: Nosematidae) infections associated with introduction of the European bumblebee in Northern Japan

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The European bumblebee, *Bombus terrestris* (L.), has been widely established throughout a broad range of Hokkaido, northern Japan since its introduction for pollinating agricultural products in 1991 and has been suggested to cause the decline of native bumblebee species. Recent invasions of *B. terrestris* into the eastern Hokkaido have been reported in 2007. The Notsuke Peninsula is covered with the species-rich maritime grassland that extends along the coast. This region is also one of the restricted distribution ranges of a rare native species, with a highly diverse bumblebee species. Given the features of the geographic region and the species involved, the invasion of *B. terrestris* into the Notsuke Peninsula is assumed to have devastating influence on native bumblebees. Here, we conducted a multi-year survey of bumblebee species to examine the population dynamics of introduced and native bumblebees. We also investigated the prevalence of *Nosema* spp. which may play an important role in the declines of native bumblebee, as well as genetic variation of the *N. bombi* rRNA ITS region for comparison with the European and North American isolates.

Poster / Microsporidia. Wednesday, 16:30. **MI-2**

Development and application of a loop-mediated isothermal amplification method for rapid detection of *Nosema ceranae*

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Loop-mediated isothermal amplification (LAMP), a novel nucleic acid amplification method, was developed for the rapid detection of the major honey bee microsporidia disease, *Nosema ceranae*. The LAMP method amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions using a set of four specially designed primers and a DNA polymerase with strand displacement activity. In this study we designed primers for LAMP assays to detect *N. ceranae* protein coding gene for DNA dependent RNA polymerase II largest subunit (RPB1) and methionine aminopeptidase type 2 (MetAP2), and evaluated the specificity and sensitivity of these assays. The detection limits for both assays was ~200

pg/µl and DNA amplification was completed within 60 min at an optimal temperature of 63°C. The assays detected 6 different geographical isolates of *N. ceranae*, and no cross-reaction was observed with other microsporidia species. The performance of LAMP and PCR was comparable: 100% specific, 100% sensitive, 100% positive predictive value (PPV), and 100% negative predictive value (NPV). In conclusion, the LAMP assay was equally specific but with a shorter detection time when compared to PCR in the identification of *N. ceranae*. The LAMP assay is an easy-to-use method and a promising alternative to conventional PCR for the rapid, cost-effective for specific identification of *N. ceranae* and other microspridia species. LAMP is considered an appropriate technology that could be used in resource-limited laboratories and the field.

Poster / Microsporidia. Wednesday, 16:30. **MI-3**

Permanent level of pathogens within ten bark beetles generations

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During the ten generations of spruce bark beetle *Ips typographus* population densities were monitored for 5-10 trap trees at several study sites in the Czech Republic in 2008-2012. On every of the four debarked section number of entry holes of spruce bark beetle were counted and then converted to density per unit area to the size of the studied sections (length about 0.5 m and about half trunk circumference). During the analysis in the field paternal beetles were collected and then stored refrigerated at -5°C. Total of 3,388 *I. typographus* beetles were dissected and checked for the presence of pathogens. In total four pathogenic organisms were detected: intestinal nematodes in 14.8%, microsporidia *Chytridiopsis typographi* in 9.1%, eugregarine *Gregarina typographi* in 0.3% and larvae of endoparasitoids in 4.9% of studied beetles. Relationship between the infection levels of pathogens and population growth of bark beetles from year to year according to the formula for calculating the rate of growth: R = logNt – logNt-1 was studied. Our research has proven that intestinal nematodes, *Ch. typographi* or *G. typographi*, did not influence the population growth of spruce bark beetle at the studied sites and are not as strong and lethal factor during the spruce bark beetle gradation. In contrast, the coefficient of population growth and the rate of beetle infested by endoparasitoids in the population is positively correlated ($y=4.72+10.38*x$; $r=0.68$; $p<0.01$; $r^2=0.47$). Parasitoids are thus able to respond very effectively to increase of the host population.

Poster / Microsporidia. Wednesday, 16:30. **MI-4**

Microsporidia in beet webworm *Loxostege sticticalis* (Pyraloidea: Crambidae): a survey of 2013

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Local populations of beet webworm in South Western Russia (populations “Slobodka”, adults sampled through May to August, “Chertkovskiy” and “Neklinosvkiy”, larvae sampled in May 2013) and Western Siberia (population “Karasuk”, sampled in June and July 2013) were examined for the presence of microsporidia. In South Western Russia, microsporidia were found only in population “Neklinovskiy”. There were three distinct microsporidian species, as proved by SSU rRNA gene sequences: *Tubulinosema* cf *loxostegi* (35% prevalence rate), *Nosema* cf *granulosis* (4%) and *Nosema ceranae* (3%). The identity of the latter species, a widespread pathogen of honey bees, was established using partial gene sequences of SSU-ITS-LSU and IGS rRNA. Its detection in a lepidopteran host implies a wider host range than though earlier and is logically explained by relatedness of *N. ceranae* to species of *Vairimorpha* which eagerly attack lepidopteran hosts and their hymenopteran parasitoids. In Western Siberia, the same isolate of *Tubulinosema* cf *loxostegi* was detected at the prevalence rates of 3% and 30% in June and July, respectively. All three species of microsporidia were able to infect beet webworm larvae in lab assays. For *Tubulinosema* cf. *loxostegi* vertical transmission to infected beet webworm progeny, experimental infection of *Galleria mellonella* and natural infection of tachina fly parasites (Diptera: Tachinidae) emerged from the microsporidia-infected beet webworm population were also confirmed.

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Poster / Microsporidia. Wednesday, 16:30. **MI-5**

Microsporidia from larvae of different lepidopteran species in Bulgaria

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Thirty-five lepidopteran species in 12 families were investigated for the presence of microsporidia in Bulgaria from April 2009 to June 2012. Infections caused by microsporidia in the genera *Nosema* and *Endoreticulatus* were identified in *Tortrix viridana, Operophtera brumata, Archips xylosteana, Orthosia cerasi, Orthosia cruda* and *Eilema complana.* The prevalence of *Nosema* spp. was low in host species: 0.3% for *T. viridana,* 2.1% for *O. brumata*, 2.4% for *O. cerasi*, 2.7% for *Archips xylosetana* and 3.3% for *O. cruda,* respectively. Spores of *Endoreticulatus* sp. were observed in 13.5% of collected *E. complana.* The spores of *Nosema* in *O. brumata* were localized in host fat body and phylogenetic studies showed that this microsporidium is relatively distantly related to .*Nosema wistmansi*, and the genera *Orthosomella* and *Cystosporogenes*. It is, however, closely related to *Nosema thomsoni. Nosema* sp. found in *Orthosia cruda* was detected in the silk glands of host larvae. Phylogenetic analysis confirmed that the microsporidium observed in the gut epithelium of *E. complana* belongs to the genus *Endoreticulatus;* however, it is not identical to other *Endoreticulatus* spp. described from Lepidoptera.

Poster / Microsporidia. Wednesday, 16:30. **MI-6**

Ultrastructural characterization of a new microsporidium (Opisthokonta: Chytridiopsida) from the pigeon feather mite *Falculifer rostratus* (Astigmata: Pterolichoidea)

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Only about 20 species of microsporidia have been described from mites. All except one species produce typical spores with a long polar filament and a polaroplast. We present the first

study of an atypical microsporidium infection in a feather mite (*Falculifer rostratus).* The infection is restricted to the *colon epithelium where it* leads to hypertrophy of the concerned cells. During sporogony multinucleate plasmodial aggregates are formed within a sporont. The sporonts are in direct contact to the host cell cytoplasm. Merogonial stages were not present. Spores are tiny (3.6 x 2.6 µm), broad ovoid in form and monokaryotic. The spore wall of mature spores has a thickness of about 240 nm and consists of a three-layered endospore and a thin, electron-dense exospore. The polar filament is anisofilar and arranged in 3–4 coils. In cross-sections it has a star-like appearance since the electron-dense core forms rounded compartments for lucent material at its surface. In grazing sections this results in a honeycomb-like pattern. A polaroplast is missing. The life cycle features and atypical spore structures clearly classify the species from the feather mite as a member of the order Chytridiopsida. Its affiliation to one of the known genera is discussed.

Poster / Microsporidia. Wednesday, 16:30. **MI-7**

Infectivity of a *Thelohania* like microsporidian isolated from *Phthonandria atrilineata* to the silkworm, *Bombyx mori*

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The pebrine of the silkworm, *Bombyx mori*, is a disease caused by infection with the microsporidium *Nosema bombycis*, also can be caused by cross-contamination of microsporidium from wild insects. We have isolated a *Thelohania* like microsporidian (TMPA) from the *phthomndria atrilineata* in the silkworm rearing region of Zhjiang province, China. The mature spores of TMPA were cylindrical or ovocylindrical in shape with a strong diopter and glossy surface. The spore size of TMPA was 3.27±0.14×2.03±0.16 µm with a length/width ratio of 1.61±0.11 µm, similar to those of *N. bombycis*. Therefore, the spores of TMPA were hardly distinguished from the spores of *N. bombycis* under light microscope. In TMPA spores formative stages, sporont produced pansporoblast including 8 nuclears by meiosis, and later 8 spores were formed in pansporoblast. Infection was systemic with mature spores produced in muscular tissue, epithelial cell of trachea, fat body, middle and posterior silkgland, fore and middle intestine, malpighian tubule and germ gland, most extensivest in muscular tissue and epithelial cell of trachea, but not in dermal cells, nerve cells, fore silkgland, posterior intestine and hemocyte cells. The IC_{50} value of TMPA to newly-hatched silkworm larvae was 1.55×10^4 spores/ml, 700-fold higher than that of *N. bombycis*, suggesting a weakly infectiousness. TMPA have transovarian transmissibility in silkworm, the rate of transovarian transmission was 1.74%, which was significant lower than that of *N. bombycis*.

NEMATODES

Poster / Nematodes. Wednesday, 16:30. **NE-1**

First release of the mermithid *Strelkovimermis spiculatus* in *Culex pipiens* mosquito populations in Argentina

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Mermithids have proved to be effective in parasitizing natural populations of mosquito larvae. However nothing is known about the inoculative introduction of this nematode in natural populations of culicids in our country. We report the results of the first field release of *S. spiculatus* in Argentine. Study area was constituted by house drainage ditches, breeding site of the mosquito *Culex pipiens* where this nematode was not present. The number and stage of mosquitoes were recorded pretreatment. *Strelkovimermis spiculatus* was introduced as second-stage juveniles (J2) obtained from laboratory cultures maintained at CEPAVE laboratory. Release was done in November 2012 (spring). A dose of 10,000 J2 per meter was applied (over a total area of 17 x 0.5 m). The number of J2 was based on previous results. Mosquito larvae were sampled 24 hs post-treatment once a week during a year, to corroborate the presence of nematode by microscopic dissection and emergence from fourth instars larvae. Parasitism by *S. spiculatus* began to be observed at third day post-application (3%). Values ranged between 0.01% and 86.3%. The highest value was recorded at 8 months post-release. This environment remained dry or without larvae during a period of four months. Nevertheless a parasitism of 45.2% was observed after this period during the first larvae collection and reaching levels between 4.8% and 86.3%. Only in three occasions was not observed infected larvae throughout the year of sampling. *Strelkovimermis spiculatus* was able to establish itself in this habitat and cause high levels of infection in *Culex pipiens* larvae.

Poster / Nematodes. Wednesday, 16:30. **NE-2**

Increased infectivity in Steinernema websteri IJ after development in desiccation-stressed hosts

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This study investigates the effect of desiccation during development on entomopathogenic nematode (EPN) infectivity. *Galleria mellonella* hosts infected with *Steinernema websteri* A10 were allowed to air-desiccate in an environmental chamber set at 23^0C for up to 31 days post-infection (DPI) resulting in a host weight loss of approximately 64%. Host carcasses were re-hydrated using reverse-osmosis (RO) water and placed in White traps to collect emergent infective juvenile populations (IJ). IJ were pooled over a three-day time period for time points on days 10, 17, 24, and 31 DPI, respectively. For a randomly chosen sample of 100 IJ for each time point, sine wave movement (number of oscillatory motions completed in one minute) and IJ morphometrics, were measured. To evaluate IJ efficacy, plexiglass “bulls-eye” traps with screens dividing sections into quadrants of specific radii were loaded using sterile soil. Twenty hosts were placed in each quadrant in the outer ring only. A dose of 10,000 IJ from each time point was placed in the center ring. Host mortality was measured over 132 hour time period. Results demonstrated that IJ collected from desiccation-stressed hosts at days 17 and 24 post-infection were significantly smaller while exhibiting greater oscillation compared with controls ($\alpha \leq 0.5$). Furthermore, efficacy experiments using bulls-eye traps demonstrated that the same desiccation-stress IJ populations killed approximately 70% of hosts between 60-72 hours post load as compared 30% mortality between 72-84 hours post load for controls. This study has implications for host delivery systems in field applications.

Poster / Nematodes. Wednesday, 16:30. **NE-4-STU**

Characterization of symbiotic bacteria *Photorhabdus luminescens* subsp. *laumondii* associated with *Heterorhabditis bacteriophora* isolated from Turkey

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The symbiotic bacteria of a novel entomopathogenic nematode *Heterorhabditis bacteriophora* isolate 48-02 was identified as *Photorhabdus luminescens* subsp. *laumondii*. This bacterial isolate did not exhibit typical signs of infection, e.g., red pigment and a gummy consistency in the host was lacking. *P. luminescens laumondii* strain 48-02 was more virulent in percentage mortality and time-to-kill compared with the molecularly similar *P. luminescens laumondii* TT01 strain. In specificity tests, *P. luminescens laumondii* strain 48-02 colonized in *H. bacteriophora* TT01 infective juvenile nematodes but the bacterial symbiont of TT01 did not colonized in *H. bacteriophora* 48-02 infective juveniles.

Poster / Nematodes. Wednesday, 16:30. **NE-5**

Pathogenicity of nematobacterial complexes and its development

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Entomopathogenic nematodes and their associated bacteria comprise together highly pathogenic complex able to invade and kill insect host within two days. Both bacteria and nematodes produce variety of factors interacting with insect immune system that help to overcome host defences. These factors are specific for each of nematobacterial complexes leading to the differences in their pathogenicity. Moreover, we observed difference in pathogenicity also between two isolates of one nematobacterial complex, *Steinernema carpocapsae – Xenorhabdus nematophila*. Ability to invade and kill insect host is low in newly emerged nematodes and develops through the time reaching its maximum after three weeks in complex *Heterorhabditis bacteriophora – Photorhabdus luminescens*. Differences in pathogenicity were observed also among particular generations of nematodes released from insect cadaver. Nematodes collected at the beginning of emergence were less pathogenic than subsequent collections. From third week of collection further we did not detect any other significant changes in nematobacterial pathogenicity, which is then influenced only by the survival of nematodes. Data describing development of infectivity and pathogenicity of *Heterorhabditis bacteriophora – Photorhabdus luminescens* complex will be used to increase efficiency and reproducibility of experimental infections used to describe immune response of insect to the nematobacterial complexes.

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Poster / Nematodes. Wednesday, 16:30. **NE-6**

Use of entomopathogenic nematodes to control vine weevils on Chilean berry orchards

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Vine weevils (Coleoptera: Curculionidae) are the most challenging pest in Chilean berry crops; they produce severe damage in the root system, decrease fruit yield and the longevity of the orchard. Besides, most of those species are quarantine pests, making obligatory their control to avoid fruit rejections in foreign markets. The control is difficult because larvae are deep into the soil or dwelling the main roots, avoiding pesticides or cultural practices. Entomopathogenic nematodes (EPN) are the most effective alternative to control these insects, because of their ability to search the larvae in the soil and even inside the dwellings. The Chilean collection of EPN (102 isolates) has been screened against the most important vine weevil affecting berries: Fuller's rose weevil *Asynonychus cervinus,* Grapevine weevil *Naupactus xanthographus*, Black vine weevil *Otiorhynchus sulcatus,* Plum weevil *Aegorhinus nodipenis* and Raspberry weevil *Aegorhinus superciliosus*. The most effective NEPs have been isolates of *Steinernema australe*, *S. feltiae* and *S. unicornum*. Average control is about 70% for these pests, measured through adult emergencies. Mass rearing has been accomplished by *in vivo* production in larvae of *Galleria mellonena* and *in vitro* through liquid media, with yields of 30-35,000 dauers/ml. NEP have been formulated in granules, gels and clays and storage up to 6 months, with 78, 80 and 72% of survivance and those dauers remain actives against the target insects. Field evaluations shows that NEP are an effective alternative for vine weevil control.

Poster / Nematodes. Wednesday, 16:30. **NE-7**

Nematodes of large larch bark beetle *Ips cembrae* (Coleoptera: Scolytinae)

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Nematodes of pest large larch bark beetle *Ips cembrae* were studied on three localities. Infestation by phoretic nematodes as well as infestation by endoparasitic nematodes in haemocel and intestinum was recorded. Phoretic nematodes were found under elytra, on wings or between body segments, especially between thorax and abdomen. It was the case of genus *Micoletzkya.* In haemocel adult females and juveniles of *Contortylenchus* sp., *Parasitylenchus* sp. and members of *Cryptaphelenchus* sp. were found. While in intestine the juveniles of *Parasitorhabditis* sp. and some tylenchid juveniles were found too. The large larch bark beetle gallery content was examined and adults of *Parasitorhabditis*, *Micoletzkya*, *Cryptaphelenchus*, *Bursaphelenchus* and *Laimaphelenchus* genera and some tylenchid juveniles were found.

This study was supported by Internal Grant Agency B0118/004 of Czech University of Life Sciences Prague.

Poster / Nematodes. Wednesday, 16:30. **NE-8**

Natural Occurence of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) in the Aydin district of Turkey

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Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are lethal parasites of insects and used for biological control of soil insect pests. Because of favorable climatic conditions, the Aydin district is an important agricultural area which produces several exported valuable crops including strawberries (mostly in grown greenhouses), peaches, citrus, chestnuts, cherries and vegetables. Each product has specific or non-specific pests in the area and farmers have difficulties to overcome some of these pests with insecticides. The objective of this study was to determine the natural occurrence of entomopathogenic nematodes in the Aydin district of Turkey. A total 83 soil samples were collected between 2011-2012 to determine the diversity and distribution of EPNs. Nematodes were isolated using the insect baiting technique. Ten EPN isolates were recovered from 83 soil samples (8.3% positive). According to morphometric and molecular analyses (28S rDNA and ITS) six of the isolates were identified as *Heterorhabditis bacteriophora* Poinar, two isolates were *Steinernema feltiae* Filipjev and one isolate was *S. weiseri* Mracek, Sturhan & Reid.

Poster / Nematodes. Wednesday, 16:30. **NE-9**

Detection of dsRNA virus-like molecules in entomopathogenic nematodes

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Viruses have been largely viewed as pathogens; nonetheless, even if most of the studied viruses are detrimental to their hosts, some of them have been reported to be also beneficial or symptomless. They are ubiquitous, and have been described infecting almost all types of organisms, from other viruses and bacteria, to animals, plants, fungi, or even protozoa. Surprisingly, little is known about viruses which naturally infect nematodes, even if they are among the most abundant animals on Earth. Nevertheless, RNA viruses infecting *Caenorhabditis* species and the soybean cyst nematode have been recently detected thanks to next generation sequencing (NGS) technologies.
Many viruses associated with persistent and symptomless infections are known to have dsRNA genomes. The presence of dsRNA molecules of sizes ranging from 1 to 14 kbp have been used as indicator of virus infection in plants and fungi. This nucleic acid can represent genomes of dsRNA viruses, as well as replicative forms of viruses with ssRNA genomes. According to this, the main objective of this work was the discovery of new viruses among a collection of entomopathogenic nematodes by using dsRNA virus-like molecules detection, which constitutes a cheaper and faster technic if compering with NGS technologies. At the present time a total of 27 strains belonging to 12 different nematode species were analyzed. Two dsRNA virus-like molecules of approximately 2.4 and 2.3 kbp were detected infecting one of the analyzed species, *Steinernema huense*. These molecules could correspond to the genome of the first identified virus infecting an entomopathogenic nematode.

Poster / Nematodes. Wednesday, 16:30. **NE-10**

Celullar and humoral interactions between the white grub, *Polyphylla adspersa* Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes

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The interaction between the white grub larvae, *Polyphylla adspersa* Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes (EPN), *Heterorhabditis bacteriophora* and *Steinernema gaseri* was addressed here. Differential Hemocyte Count (DHC) for the both second and third instar larvae of the white grub, granulocytes (65.25± %2.22) and plasmatocytes (22.14± %1.14) were most abundant cell types in the circulating hemolymph. Study on hemocyte and humoral reactions of the white grub larvae against the EPNs was performed by injection 20 monoxenic infective juveniles (IJs) of *S.glaseri* and *H. bacteriophora* into the insect hemocoel. The hemocel of the larvae at different hours post injection (hpi) was dissected and showed changes in total hemocyte count (THC), DHC and cell shape. Encapsulation was a typical cellular reaction, which its maximum rate was observed by 8 hpi of *H. bacteriophora* and 12 hpi of *S. glaseri.* The encapsulation reaction in third instar larvae was observed stronger than those of the second instar larvae. Also the encapsulation reaction against the *H. bacteriophora* had significant different with those against *S. glaseri*. In contrast to second instar larvae, third larval stage had higher specific phenloxidase activity when challenged with both EPNs species. It was showed the defense system could create initial melanization at 18 hpi of *S. glaseri* and 12 hpi of *H. bacteriophora.* However, EPNs probably reduced the hemocyte number in circulating hemolymph by their symbiotic bacteria. This occurrence which followed by reduce in THC level decreased the cellular and humoral intensity response of the larvae. Therefore, the immune system of the grub was suppressed by the EPNs while this system was activated in early stage of infection. This study showed weak immunity response of the white grub larvae of *P. adspersa* against EPNs, *S. glaseri* and *H. bacteriophora*. This finding could be helpful for the pest management by select the suitable EPN species in term of virulnce and ability to suppress the insect defense system.

Poster / Nematodes. Wednesday, 16:30. **NE-11**

***Oscheius rugaolensis*, new genus and species of insect parasitic nematodes from Iran**

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During 2013, a survey was carried out to determine the pathogens of *Polyphylla adspersa* (Col; Scarabaeidae) in the Mashhad region, North East of Iran. All larval instars of *P. adspersa* with sign of nematode infection were collected and death larvae were transferred to the White trap. By using this method fifteen nematode isolates were isolated. Among the pathogenic agents, entomophatogenic and parasite nematodes had a moderate frequency. The initial identification of the collected nematodes carried out using morphometric data. Subsequently, molecular identification and phylogenetic analysis were performed using DNA sequences of ITS and 18SrDNA genes. The molecular data indicated that wg10 and wg19 isolates belong to *Oscheius* genus with 99% bootstraps support. Also, Nblast analysis introduced two isolates wg10 and wg19 as *O. rugaolensis*. The sequence of 18S gene *O.*

rugaolensis differed with wg10 and wg19 in 8 and 1 nucleotides, respectively. While on the basis of ITS sequences, 7 nucleotides were differed. The phylogenetic relationship was analysed based on bayesian procedure. In the reconstructed phylogenetic tree, wg10 and wg19 isolates were placed together with *O. rugaolensis* in a clade by 100% bootstraps support. The phylogenetic results from both genes, ITS and 18S, were similar. This is the first report of *Oscheius* genus for Iran. Despite the free living behavior of this species, it had high virulence on some insect species and higher ability to reproduce on the cadavers of *Galleria melonella* rather than healthy larvae. Future studies may provide more data about ability of this species as biocontrol agent.

Poster / Nematodes. Wednesday, 16:30. **NE-12**

Reproduction status of *Tribolium castaneum* affects its response to infection by *Steinernema feltiae*

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Gender specific reproductive roles are a reason of sexual dimorphism not only in a body size but also in a whole range of physiological traits. We investigated differences between sexes as well as reproduction status (virgin vs. reproducing) of the red flour beetle, *Tribolium castaneum* in defence against infection by the nematode, *Steinernema feltiae*. Females and males of the beetles either virgin or after copulation were exposed individually to the nematodes. The beetles during infection were kept without food. From each group 20 individuals were sampled after 12, 24, 36 and 48 hours. Ten individuals of each sample were dissected and checked for the presence of the nematodes, ten were frozen for further phenoloxidase activity measurements.
Reproduction strongly affected the response of females – they mortality and parasite load was the highest among all studied group. This group had also the lowest phenoloxidase activity. At the same time, we did not observed differences between virgin beetles as well as between virgin and reproducing males. Surprisingly, eggs production itself did not increase females vulnerability to parasite – we observed eggs also in the body cavity of virgin females. Probably production of unfertilized eggs is less expensive than fertilized ones. The highest parasite load we found just after infection and after 48 hours. Last outcome can be explained by starvation of the beetles so they were weakened and the nematodes more easily infected them. Our results confirm that cost of reproduction may impair defence mechanism and immunological system of *T. castaneum* females..

Poster / Nematodes. Wednesday, 16:30. **NE-13**

Effect of culture type, container type, and temperature on a Korean strain of the entomopathogenic nematode, *Steinernema carpocapsae*

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A Korean isolate of the entomopathogenic nematode (EPN) *Steinernema carpocapsae* KCTC 0981BP strain (ScK) is effective for control of many Korean agricultural and forestry pests. In vitro culture is available for large scale of mass production of commercial EPN, but it is a costly and complicated process, whereas in vivo culture using great wax moth *Galleria mellonella* larvae is simpler for small scale production. However, culture type and storage temperature during in vivo culture may influence harvesting and survival of EPN. We investigated effects of those factors on harvest, survival, and pathogenicity of ScK. Storage period, culture method, and storage container and temperature all influenced ScK survival. ScK survived better in small cultures rather than in mass culture, and better in Zip-lock containers than in tissue culture container. The best storage temperatures for ScK were 13 and 20℃ in small scale culture while there were no differences among temperatures in mass culture. The highest yields of ScK were obtained by rearing them in small cultures and keeping them in Zip-lock containers at 20 or 13℃. The pathogenicity of ScK differed among treatment combinations on the 1st day after inoculation, but there were no differences on the 3rd day. The number of established nematodes differed depending on storage temperature and period.

Poster / Nematodes. Wednesday, 16:30. **NE-14**

Steinernema feltiae (Nematoda: Steinernematidae) to control fungus gnat, _Bradysia mabiusi_ (Diptera: Sciaridae): effect of dosage and application time*

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Fungus gnat, *Bradysia* spp., is a pest of worldwide importance for nursery plants. The larvae feed on the roots of emerging plants, becoming potential target to use of entomopathogenic nematodes. This study aimed to determine the best time for the application of *Steinernema feltiae* after the exposition of the substrate (inside the pot) to the insect adults. Sixteen treatments were considered: the nematode applied at three doses 3, 14 and 70 IJs/cm^2 (173, 883 and 4417 IJs/pot, respectively), applied soon after the infestation of the substrate with adults, as well as by 7 days, 14 and 21 days after, plus the respective controls. For each treatment, four replications were considered, with each replication composed by a plastic pot (200 ml) containing 50 g of substrate (10% humidity) and 3 grains of black bean (pre-cooked) gathered on the substrate surface, on the center of the pot, for larval feeding. The pots were transferred to inside of a large cage containing the insect rearing and exposed to the adult population for 2 hours to allow insect oviposition. Then, the pots were transferred individually to inside of other chambers (1 liter) containing a double yellow plastic sheets (8.0cm x 8.0cm) covered with insect glue for attracting and capture the emerging adults. The best time for application of the nematode was 3 weeks after the exposition of the substrate to adults, providing 61, 69 and 78% control for the doses of 3, 14 and 70 IJs/cm^2.

Poster / Nematodes. Wednesday, 16:30. **NE-15**

The non-sterilizing strain of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) and its development on different strains of *Amylostereum* (Basidiomycota: Russulales)

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The nematode *Deladenus siricidicola* Kamona, which sterilizes *Sirex noctilio* females, has been extensively and successfully used as a biological control agent for this woodwasp in the Southern Hemisphere. Curiously, a non-sterilizing (NS) strain of *D. siricidicola* is commonly found in North America and it is thought that the NS strain was introduced with *S. noctilio* when *S. noctilio* was introduced to North America. Finding an appropriate biological control agent in North America has been challenging due to the existence of native species of *Sirex* woodwasps that are not considered pests but are part of the decomposer community in forests. Therefore, evaluation of biological control agents requires studies of host specificity of the nematodes. For this experiment, we evaluated the NS strain of *D. siricidicola*, which is poorly understood and is a potential competitor of *D. siricidicola* Kamona. *D. siricidicola* has two forms: a form that parasitizes *S. noctilio* and a mycophagous form that feeds on the fungal symbiont of *S. noctilio, Amylostereum*. The goal of this study was to investigate associations between the NS nematodes and different isolates of the symbiotic fungus, mainly to evaluate the ability of the nematodes to develop and reproduce on different isolates of *Amylostereum* associated with *Sirex* in North America.

Poster / Nematodes. Wednesday, 16:30. **NE-16**

Use of entomopathogenic nematodes in the biological control of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae)

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The gypsy moth is one of the major insect pests, commonly distributed in west Georgia. Insect damages mainly foliage trees and spread easily from forest vegetation to fruit orchards. The aim of the research was to study efficacy of two species *S. carpocapsae* and local *S. thesami* against larvae of the gypsy moth in field conduction. Nematodes were reared produced in vivo, *Galleria mellonella* larvae. (Temperature=23ºC and hygrometry=88-92%). Experiments against larvae of the gypsy moth were carried out in June, in the area adjacent to the deciduous forests of the Tbilisi National Park. Small, young crab-apple and wild pear trees were chosen for experiment. The average number of pest specimens on 1 m^2 branch of the each experimental plant was 74.3±4; 58.6±5; 85.2±6 and 78.3±5 on the control plant. About 30 liters of nematode suspension was used to treatment of experimental trees. One part of plants was treated with *S. carpocapsae* suspension 1500 IJs/ml of water, and the second part with the same dose of *S. thesami.* Experiments on the same pests were performed with increased concentration - 3000 IJs/ml of water.
The calculation of the insect mortality in field conduction was carried out on the 7^{th} day after treatment. The larval mortality rate was 77.5% - 63.3% where low concentration of nematodes was used. In the case of double concentration mortality was 88.6 and 76.3% respectively.
On the basis of the results obtained it can be noted that *S.carpocapsae* proved to be more efficient (10-12%) compared with the local species *S.thesami*.

Poster / Nematodes. Wednesday, 16:30. **NE-17**

The susceptibility of Colorado potato beetle *Leptinotarsa decemlineata,* and mulberry moth *Glyphodes pyloalis* to entomopathogenic nematodes, *Steinernema carpocapsae* and *Steinernema feltiae* in Georgia

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Colorado potato beetle, *Leptinotarsa decemlineata* and mulberry moth, *Glyphodes pyloalis* are the major pest insects of vegetable and urban horticulture crops in Georgia. The aim of this study was to determine the efficacy of entomopathogenic nematodes *Steinernema carpocapsae* and *Steinernema feltiae* against *L. decemlineata* and *G. pyloalis* larvae under laboratory and field conditions. In the laboratory, *S. carpocapsae* and *S. feltiae* caused 92% and 62% larval mortality on *L. decemlineata,* respectively. *S. carpocapsae* also caused high mortality (74%) than *S. feltiae* (52%) in the field study. For *G. pyloalis*, *S. carpocapsae* induced greater larval mortality (82 and 72%) than *S. feltiae* (65 and 61%) under the laboratory and field conditions, respectively. In conclusion, *S.carpocapsae* exhibited significantly greater efficacy than *S. feltiae* against both insect species. The results suggest that *S. carpocapsae* has a great biological control potential against *L. decemlineata* and *G. pyloalis* larvae in Georgia. However, the efficacy of *S. carpocapsae* should be tested in large-scale field studies.

Poster / Nematodes. Wednesday, 16:30. **NE-18**

Co-infection interactions between entomopathogenic fungi and *Steinernema feltiae* using*Tenebrio molitor* as a model system

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Prior studies have been conducted investigating additive, synergistic, or antagonistic interactions between multiple types of biocontrol agents when co-infecting an insect host. Fewer studies have focused on combining entomopathogenic nematodes (EPNs) and entomopathogenic fungus (EF) to control weevils and scarab grubs. None of these studies have investigated interactions between *Steinernema feltiae* and EF. The present study investigates co-infection interactions between commercially produced *S. feltiae* and two isolates of EF, using *Tenebrio molitor* (Coleoptera) as a model host system. *T. molitor* larvae were infected with either *Beauveria* or *Metarhizium* isolated from naturally infected insects collected in strawberry fields in Denmark. At different intervals following EF infection, larvae were exposed to S. *feltiae*. The impact of fungal infection on the nematode was measured by counting the number of infective juveniles that penetrated the host in comparison to the number of infective juveniles that penetrated control larvae with no prior EF exposure. Daily mortality was recorded, and cadavers from nematode treatments were monitored for mycosis and placed on white traps in order to compare the total number of *S. feltiae* offspring produced in the presence of fungal infection. We discuss the use of *T. molitor* as a model system and the extrapolation of these results for the control of strawberry blossom weevil, *Anthonomus rubi*.

Poster / Nematodes. Wednesday, 16:30. **NE-19**

Some observation on morphology and ecology of mollusc-parasitic nematode *Alloionema appendiculatum*

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Alloionema appendiculatum is a common larval parasite of many terrestrial molluscs. Its 3rd stage larvae (dauer juveniles) invade foot muscle of snails and slugs. Dauer juvenilles

develope in to the 4th stage larvae, that leaves slugs. Later they mature and reproduce in the soil. Despite the fact this nematode is a parasite of snails in helicicultre and also an invasive slug *Arion vulgaris* (syn. *A. lusitanicus*), that is one of the most serious pest in agriculture and horticulture, the knowledge about morphology and ecology of this nematode are very poor. We performed some studies of this nematode with a goal to provide new information about morphology, phylogeny and ecology of this species. This work brings, above all, the complete redescription of *A. appendiculatum*, include molecular biological characterisation suggesting high intraspecific variabily in ITS region. Results of ecological studies provided new information about the saprobic life cycle and natural prevalence, but also show that, in standard conditions, *A. appendiculatum* has very week influence on mortality and feeding activity of slugs *A. vulgaris*, while in other stressful conditions it might be an important agent controling population density. But we concede that this can be also strongly influenced by bacterial associates, even though the role of bacteria in nematode development is questionable.

Poster / Nematodes. Wednesday, 16:30. **NE-20**

Osmotic stress tolerance and infective juvenile production of entomopathogenic nematodes subject to fast host-desiccation treatments

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Entomopathogenic nematodes (EPN) are being used commercially in several countries for the control of soil dwelling pests. However, their effectiveness is affected by environmental stresses such as low soil moisture. An alternate method for ensuring nematode's survival and infectivity is to apply them in the cadavers of *Galleria mellonella* used to reproduce them. It has been reported that the IJ's emerging from cadavers have increased infectivity and higher tolerance to low soil moisture and high temperatures. To determine the optimum time post infection and intensity of desiccation for higher IJ's production and their effects on osmotic stress tolerance in these EPN a laboratory experiment was carried out. Our results showed that timing to start desiccation (2, 4 and 6 days post-infection) and intensity (1, 2 and 4 days in a desiccator) affected weight reduction, especially in *S. glaseri*, which resulted in higher death rates of the IJ's. The total number of nematodes, however, was not related to the opportunity or intensity of the stress treatments, but to nematode species and initial weight of the hosts. In an evaluation of survivorship in a 30 % PEG-8000 solution, pre-conditioned *Heterorhabditis bacteriophora* showed a significantly higher tolerance to osmotic stress than *Steinernema glaseri* and showed an increase in tolerance 100 % larger than the observed with the last nematode species. The higher percent of survivorship was obtained with IJ's from hosts where desiccation treatments initiated 2 days post-infection in both EPN.

Poster / Nematodes. Wednesday, 16:30. **NE-21**

Assessing entomopathogenic nematode population genetics: a research and teaching approach

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While entomopathogenic nematodes (EPN) are important components of ecosystems, relatively little is known about the genetics of individual EPN populations in natural settings. We are combining an attempt to answer the question "How related are EPN found in natural settings?" with an integration of EPN into an undergraduate Genetics course module on population genetics. We used Random Amplified Polymorphic DNA (RAPD) approaches, and are working with lab maintained geographic isolates of EPN to identify appropriate primers and develop methodology. We have tested our technique by first assessing the genetic variability of a single geographic isolate of a single EPN species, and then exposed waxworms to a combination of geographic isolates of that species. We then assessed the genetic variability of the IJs that emerged from "mixed-isolate" waxworms. RAPD has been effective at identifying markers for individual geographic isolates, and for assessing the population genetics from "mixed-isolate" populations. RAPD is also a standard technique taught in Genetics labs, meaning that a high throughput of samples is possible and that undergraduates are exposed to real-world questions in the classroom. Once this technique has been fully developed for laboratory isolates, we plan to move this research effort into the local ('natural') environment, where we will answer the original question regarding the population genetics of local EPN isolates pre- and post-infection. This may improve our understanding of how natural populations are structured, and hopefully will provide insight that is relevant to the use of these organisms for biological control.

VIRUSES

Poster / Viruses. Wednesday, 16:30. **VI-1**

High-level Expression of Foreign Protein Using the Partial Polyhedrin-fused Baculovirus Expression System

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Polyhedrin is the major component of the nuclear viral occlusions produced during replication of the baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). To enhance the production efficiency of foreign protein in baculovirus expression system, the effects of various polyhedrin fragments were investigated by fusion expressing them with the enhanced green fluorescent protein (EGFP). Recombinant viruses were generated to express EGFP fused with polyhedrin fragments based on the previously reported minimal region for self-assembly and the KRKK nuclear localization signal (NLS). The marked increase of EGFP production by these fusion expressions was confirmed through protein and fluorescence intensity analyses. Among the fusion-expressed protein in nucleus and cytoplasm, the most hyper-expression was observed in the fusion of amino acids 19 to 110 and 32 to 59 of polyhedrin. The marked increase of production of several other foreign proteins was proved by the fusion expression with these polyhedrin fragments. This study suggests a new option for higher expression of useful foreign recombinant protein by fusion expression with the partial polyhedrin in baculovirus.

Poster / Viruses. Wednesday, 16:30. **VI-2**

A natural recombinant between *S. frugiperda* MNPV and *S. litura* NPV

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A Colombian SfMNPV isolate (SfCOL) and its genotypic variant (SfCOL-A) have shown great potential as active ingredients for a biopesticide product to control the fall armyworm *S. frugiperda* in Colombia. The complete genomic sequence of SfCOL-A was determined and analyzed in the present study, consisting of 134,239 bp, encoding 144 putative open reading frames. Gene synteny maps showed great colinearity with genomes of other sequenced SfMNPVs. SfCOL-A genome displayed a ~1470 bp deletion localized within the main variable region among SfMNPV geographical isolates and their genotypic variants previously described. Interestingly, a ~2970 bp sequence block insertion, carrying two ORFs which lacked any similarity with previously described SfMNPV genes, was also found in this region.The highest identity values and codon usage similarity within the inserted sequence suggested the idea of a recombination event between SpltNPV-II (or a similar virus) and a wild type Colombian SfMNPV. Two bioinformatics approaches (relative similarity and bootscanning analysis) were used to explore the recombination hypothesis. Both analyses supported the hypothesis, showing a recombination event involving the C_{term} region of the *chitinase* ORF and the N_{term} region of the *gp37* ORF. This event resulted in the deletion of a genomic region including the *Homologous Region 3* (HR3) and the *Sf23* ORF; and an insertion of ~2970 bp carrying the splt020 and splt021 ORFs from SpltNPV-II. Breakpoints seemed to be localized within the frames of *chitinase* (near position 21,471) and *gp37* (near position 24,443) genes in SfCOL-A, restoring the integrity of both frames. These results suggested a natural recombination between heterologous baculoviruses involving genes that encode non-essential proteins and affect the viral phenotype.

Poster / Viruses. Wednesday, 16:30. **VI-3**

Host specificity and PIFs based phylogeny of Betabaculovirus isolates from Gelechiidae family

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Tecia solanivora, *Phthorimaea operculella* and *Tuta absoluta* are insect species belonging to the potato tuber moth complex (Lepidoptera: Gelechiidae) and are considered the main pests of potato and tomato crops. In this sense, baculovirus constitutes a useful tool for the biological control of these insects. In the viral cycle, a protein complex known as PIF (*Per Os* Infectivity Factors) are responsible for the virus entry into the mid gut cells determining the host range. In the present work the heterologous host infection of three viral isolates (recovered from moths of each insect species *T. solanivora, P. operculella* and *T. absoluta*) was determined by oral inoculation of each specie larvae with occlusion bodies (OBs). Infection by each of the three Granuloviruses in the three different host species produced a fatal disease. Additionally, comparative sequence analysis of *pif* genes was assessed. Seven pairs of degenerated primers were designed to amplify sequences of *p74*, *pif1*, *pif2*, *pif3*, *odv-e28*, *odv-e56* and *pif6*. The PCR products were cloned and sequenced. Comparative analysis of *pif* sequences of three isolates revealed high similarity with *Phthorimaea operculella* granulovirus (PhopGV) previously reported in the Genbank. The topology of phylogenetic tree using concatenated deduced aminoacid sequence of seven PIFs was consistent with previously published trees for Baculoviruses using three conserved genes or complete genomes. The three isolates evaluated were grouped with PhopGV. These results suggest a potential use of Granuloviruses isolated from different species of Gelechiidae family for biological control in heterologous species and showed the utility of *pif* (core genes) for phylogenetic studies in Baculoviruses.

Poster / Viruses. Wednesday, 16:30. **VI-4**

Diagnosing the unknown – advancing the taxonomy of aquatic invertebrate viruses

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Few viruses from marine invertebrates, in particular crustaceans, have been assigned to a virus family with certainty because biochemical, biophysical and immunological data are incomplete or lacking, essentially due to the lack of crustacean cell cultures. Particularly, we have very limited information on viral infections in non-commercial crustacean species or in other invertebrates that may be living in the same environment. Crustacean viruses have so far been tentatively assigned to families based upon morphological and developmental characteristics and the location within the cell. The need to complete full characterisations and harmonise the naming of new viruses using International Committee on Taxonomy of Viruses (ICTV) guidelines is evident throughout the literature with many different names or abbreviations being used to describe the same virus. We present the identification of a virus infection from wild caught *Crangon crangon* (brown shrimp), the optimisation of viral purification techniques from these samples, and the application of next generation sequencing to characterise the viral genome. Similarities between crustacean viruses and those described in other invertebrates including insects may assist in classification of this novel virus. The data obtained will be also be used to develop a diagnostic tool.

Poster / Viruses. Wednesday, 16:30. **VI-5**

Proteomic analysis of the occluded Tipula oleracea nudivirus (ToNV)

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The *Nudiviridae* family has been recently established by the International Committee on Taxonomy of Viruses (ICTV). Although six fully sequenced genomes are now available in databases and protein profile from nudivirus particles were mainly characterized by polyacrylamide gel electrophoresis, only few direct matches have been published between genomic and proteomic data to the exception of the major occlusion body protein (mOBp) from Penaeus monodon nudivirus (PmNV) and four nucleocapsid proteins from Helicoverpa zea nudivirus 2 (HzNV-2). Function of nudiviral predicted proteins is still inferred from what is known from their baculovirus sister-group and the occluded nature of virions remains incidental to the *Nudiviridae* family. Tipula oleracea nudivirus (ToNV) is one of the causative agents of crane fly nucleopolyhedrosis. The dsDNA virus genome was recently sequenced. Phylogenetic analysis revealed ToNV is related to Betanudivirus clade representatives and distantly related to another Diptera-infecting nudivirus representative, the Drosophila innubia nudivirus (DiNV). Electronic microscopies showed occlusion bodies are irregularly shaped and measure from 2 to 5 µm in length and 2 µm in mid-diameter. They are filled with rode-shape virions containing a single nucleocapsid within a tri-layered envelope. Quantitative proteomic analysis using on-line nanoflow liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) revealed ToNV occlusion bodies are composed of 47 viral proteins, of which the most abundant are the functional homolog of baculovirus major occlusion bodies proteins and the homologs to two HzNV-2 predicted ORFs corresponding to virion structural proteins.

Poster / Viruses. Wednesday, 16:30. **VI-6**

Nucleopolyhedrovirus and Microsporidia in Winter Moth (*Operophtera brumata, L.*) and Bruce Spanworm (*O. bruceata, Hurst*) populations in the Northeast US

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The winter moth (WM, *Operophtera brumata, L.*), a polyphagous geometrid affecting mainly deciduous tree species was accidently introduced to the Northeast United States from Europe in the 1990s. Although WM has been in a continuously outbreaking population since its introduction, the native congener, Bruce spanworm (BSW, *O. bruceata, Hurst*) rarely exhibits outbreaks. We propose that this difference in population dynamics exists because BSW is experiencing a different set of pathogens, which exist at a higher prevalence. Field collected WM and BSW larvae were reared in the lab and percent mortality was noted. Cadavers were examined microscopically for evidence of *Microsporidia* and nucleopolyhedrovirus (NPV) infections. DNA was extracted from BSW samples that were positive for NPV, and amplified by PCR to detect and characterize polyhedrin gene sequences. Of 433 BSW larvae, 51.5% did not survive to the pupal stage while only 1.1% of the 15,677 WM larvae died prior to pupation. BSW had a higher prevalence of Microsporidian infection than WM (63.0% compared to 3.3%) while WM experienced a high prevalence of NPV (93.3% compared to 14.1%). Polyhedrin sequence from BSW was only 88% identical to that of OpbrNPV, indicating that the NPV infecting these insect species are different. In conclusion, WM and BSW are experiencing different pathogens and at a different prevalence. Understanding the controls of epizootics on BSW may provide valuable insight into possible biological controls for WM.

Poster / Viruses. Wednesday, 16:30. **VI-7**

Regulation and activation of two effector caspases that affect Sindbis virus replication in *Aedes aegypti* mosquitoes

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The yellow fever mosquito (*Aedes aegypti*) proteins CASPS18 and CASPS19 are closely related effector caspases that are hypothesized to be involved in midgut escape of Sindbis virus (SINV). Silencing CASPS18 and 19 expression results in decreased SINV titer in *A. aegypti* following oral infection, while overexpression of CASPS19 by recombinant SINV in *A. aegypti* causes increased virus replication. Furthermore, levels of the midgut basal lamina proteins collagen IV and laminin are increased in infected mosquitoes when CAPS18/19 are silenced, and decreased by overexpression of CASPS19, consistent with a role in midgut escape. CASPS18 lacks a typical caspase active site motif (QACRG) and has no enzymatic activity, but is able to directly enhance the activity of CASPS19. To investigate the mechanism of enhancement, we examined whether the two proteins interact and found that CASPS18 co-immunoprecipitated with CASPS19 when expressed in Sf9 cells. Under these conditions, both CASPS18 and 19 underwent a proteolytic processing event that released the small subunit. The intact CASPS19 catalytic site was required for processing of both proteins. Recombinant purified CASPS18 enhanced the activity of purified active CASPS19 in vitro, but was not able to induce the activation of unprocessed CASPS19, indicating that the enhancement of CASPS19 activity by CASPS18 occurs after CASPS19 activation. Recombinant CASPS19, alone or with CASPS18, could not directly cleave collagen IV or laminin, suggesting that the effect of these caspases on the levels of midgut basal lamina proteins is not through direct cleavage, but may instead be an indirect effect.

Poster / Viruses. Wednesday, 16:30. **VI-8**

Proteomic analysis and *in vivo* diferential gene expression of *Trichoplusia ni* granulovirus (TnGV)

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Nucleopolyhedrovirus (NPV) and *Granulovirus* (GV) infections on *Trichoplusia ni* larvae is regulated by the expression of the virus genes. There are very few reports about the differential expression of the baculovirus genes in the host. This report deals with the proteomic analysis to detect the differential expression of viral genes. Also, macroarrays were prepared to analyze total proteins from infected *T. ni* larvae with the granulovirus TnGV, which were compared with those from non-infected larvae, obtained at different post-infection (p.i.) periods. When the expressed proteins from infected and non-infected larvae were compared, no significant change in the protein pattern was observed at 24 hs p.i.; however, when compared at 48, 72, 90, and 120 hs p.i., differential protein bands were detected in the infected larvae, not present in the non-infected larvae. Additionally, substractive libraries were constructed in order to identify those genes expressed differentially at different p.i. periods. Libraries were obtained with 36, 21, 16, 13, and 23 clones at 24, 48, 72, 96, and 120 hs p.i., respectively. In these macroarrays a decrease of the hybridization intensity was observed as the p.i. periods were increasing. This observation may suggest that, due to the TnGV infection in the *T. ni* larvae tissues, the expression of normal proteins of the host decreased. That is, there might be an expression repression of the larval

genes. This report it sets the bases to understand the induction and repression mechanisms of the insect genes, when a GV infection occurs..

Poster / Viruses. Wednesday, 16:30. **VI-9**

Recombinant Iridovirus IIV-6 expresing the Cn-10 neurotoxin from *Centruroides noxius* scorpion

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Was established the methodology to obtain a recombinant Iridovirus *in vivo,* using the microparticle bombardment. Genes encoding for the green fluorescent protein GFP, and the protein Cn10, was cloned into the 295L gene Iridovirus, strain IIV -6. Were standardized optimal conditions for micro-projectile bombardment cotransfection of the vector DNA TOPO-295L-GFP- Cn10 and wild DNA from Iridovirus IIV-6, being the ratio of 3:1 (vector: wild DNA) the more useful for obtained recombinant Iridovirus. Recombinant Iridovirus IIV-6, was obtained by co-transfection of vector DNA TOPO-295L-GFP-Cn10 and wild DNA Iridovirus IIV-6, using the technique of biolistic to co-infecting *Galleria mellonella* larvae. This recombinant Iridovirus expressed both proteins, the GFP and Cn10. Furthermore, using fluorescent microscopy, were detected a green fluorescent staining in few portions of fat tissue of *G. mellonella* larvae. The potential expression of GFP and Cn10 proteins, was corroborated by SDS-PAGE, restriction analysis and PCR. This is the first report of the production of a recombinant Invertebrate Iridovirus, expressing a reporter gene (GFP) and a virulence gene (Cn10) and represents a model system for the genetic improvement of Invertebrate Iridovirus. More studies are needed at the molecular level, such as the sequencing of the genome of recombinant Iridovirus IIV-6 and performing Western Blot tests to detect, for one hand, the insertion of both genes (GFP and Cn10) into the genome of IIV-6 Iridovirus, and on the other hand, to verified the correct expression of both proteins in the tissues of the infected insect larvae..

Poster / Viruses. Wednesday, 16:30. **VI-10**

Genomic sequencing and analysis of Sucra jujuba nucleopolyhedrovirus

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The complete nucleotide sequence of Sucra jujube nucleopolyhedrovirus (SujuNPV) was determined by 454 pyrosequencing. The SujuNPV genome was 135,952 bp in length with an A+T content of 61.34%. It contains 131 putative open reading frames (ORFs) covering 87.9% of the genome. Among these ORFs, 37 were conserved in all completely sequenced baculovirus genomes, 25 conserved in lepidopteran baculoviruses, 64 were found in other baculoviruses, and 5 were unique to SujuNPV genome. Seven homologous regions (*hrs*) were identified in the SujuNPV genome which can be classified into two groups. SujuNPV was identified to contain several duplicated or multiple copy genes, as it contains two copies of helicase, DNA binding protein gene (*dbp*) and *cg30,* 3 copies of inhibitor of apoptosis gene (*iap*), and 4 copies of baculovirus repeated ORF (*bro*). Phylogenetic analysis suggest that SujuNPV belongs to a subclade of group II alphabaculovirus, interestingly different from other baculoviruses, all the nine members of this subclade contain a second copy of *dbp*.

Poster / Viruses. Wednesday, 16:30. **VI-11**

Functional analysis of exonuclease gene (012L) of *Chilo* iridescent virus

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Chilo iridescent virus (CIV) encodes an open reading frame (ORF 012L) homologous to exonuclease II of *Schizo-saccharomyces pombe*. In the current study, we focused on the characterization of *exonuclease* gene of CIV. The target gene was cloned into the pET28a vector, expressed in *E. coli* strain BL21 (DE3) Lys with an N-terminal His tag and purified to homogeneity by using Ni-NTA affinity chromatography. Biochemical characterization of the purified CIV-exonuclease protein (CIV-Exo) confirmed that this viral protein is a functional 5'-3' exonuclease that digests 3'-biotin-labelled oligonucleotides and linear double-stranded DNA molecules from their 5'-termini in a highly processive manner. CIV-Exo has also a potent endonuclease activity in vitro. The CIV-Exo converted supercoiled plasmid DNA (replicative form I, RFI) into the open circular form (RFII) and then open circular form into linear form (RFIII). Both exonuclease and endonuclease activities of CIV-Exo are optimal at pH 8.0 in the presence of 10 mM $MgCl_2$, 2 mM dithiothreitol and 100 µg BSA ml^{-1}.

Poster / Viruses. Wednesday, 16:30. **VI-12**

Identification of a new multiple nucleopolyhedrovirus isolated from the Jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Egypt

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A new multiple nucleopolyhedrovirus was isolated from diseased larvae of the jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Egypt. The virus caused typical symptoms of a baculovirus infection, and it was possible to propagate the causative agent in larvae of the homologous host. Light microscopy studies showed polyhedral occlusion bodies (OBs). Electron microscopy of ultrathin sections of polyhedral OBs showed multicapsid virions identifying the virus as a multiple embedded nucleopolyhedrovirus. Therefore, this virus was termed *Palpita unionalis* multiple nucleopolyhedrovirus (PaunNPV). The identity of the isolated virus was confirmed by sequencing of a 452 bp fragment of the *polyhedrin (polh)* gene that was amplified using degenerate primers. Blast search showed that it was closely related to *polh* genes in *Dirphia peruvianus* NPV, *Pterolocera amplicornis* NPV, and *Nepytia phantasmaria* NPV. A neighbour-joining phylogenetic tree was constructed based on the predicted amino acid sequences of the *polh* genes of the selected closely related NPVs. Phylogenetic distances suggested that PaunNPV should be considered to belong to a novel species within the genus *Alphabculovirus*. Preliminary bioassay data showed that the virus was active against either 2nd or 4th instars of jasmine moth. The calculated

LC_{50} was $1.3x10^3$ and $3.1x10^3$ OBs/ml for the tested 2nd and 4th instars, respectively. The study reports a new baculovirus that might be used as a promising agent for biological control of the jasmine moth.

Poster / Viruses. Wednesday, 16:30. **VI-13**

A single baculovirus for the production of recombinant Adeno-Associated Virus 8 vectors

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We have developed a single baculovirus, named "Monobac", for the production of recombinant Adeno-Associated Virus vectors of serotype 8 (rAAV8) using the Sf9 cell/baculovirus system. In an AcMNPV bacmid devoid of the *chitinase* and *cathepsin* a genes, the AAV *rep2* and *cap8* genes have been inserted at the *egt* locus, while the recombinant AAV was cloned in the Tn7 site. This system was used for the production rAAV8 encoding the human γ-sarcoglycan gene, of clinical interest for the treatment of LGMD2C (γ-sarcoglycanopathy) myopathy disease. Enhanced rAAV8 productivity was observed in the cell culture and was maintained after purification, compared to production system based on the use of 2 baculoviruses. The produced rAAV8 capsids displayed a reduced degradation profile of the capsid proteins VP1/VP2 due to the elimination of the baculovirus *cathepsin* protease gene. This optimized system allows the production of an improved quantity of rAAV vectors with improved vector quality, resulting in enhanced infectivity of the rAAV. .

Poster / Viruses. Wednesday, 16:30. **VI-14**

Determining the role of P10 during baculovirus infection through the development of novel mutants in *Autographa californica* multicapsid nucleopolyhedrovirus

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P10 is a fibrous protein that forms complex networks of filaments and a distinct perinuclear tubular structure around the nucleus during the later stages of infection of cells with baculovirus. Previous research has suggested possible roles of P10 in nuclear stability, polyhedron formation and cell lysis, but distinct functional roles for the protein have yet to be determined. In order to investigate the role of P10 during infection, a variety of *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) mutants have been constructed, which include a *p10* deletion and associated rescue virus, phosphorylation mutants and a virus in which the AcMNPV *p10* coding region has been replaced with that from *Spodoptera frugiperda* (Sf)NPV. Mass spectrometry was used to confirm the phosphorylation of P10 serine 93. Mutation of serine 93 to alanine affected the structure of P10 tubules as evidenced by confocal microscopy. The distinctive tubular structure surrounding the nucleus that is observed in wild-type virus infected cells failed to form correctly. Circular dichroism analysis confirmed a distinct change in the protein secondary structure. These data suggest that phosphorylation plays a key role in P10 function. Replacement of the AcMNPV *p10* coding region with that from SfNPV resulted in a virus with low budded virus titre and aberrant rearrangement of microtubules in comparison to AcMNPV-infected cells, suggesting that the SfNPV P10 may be affecting microtubules and translocation of nucleocapsids to the plasma membrane for budding.

Poster / Viruses. Wednesday, 16:30. **VI-15**

Evaluation of the transcriptional transactivation of betabaculovirus regulatory elements in transformed cell lines by alphabaculovirus transcription factors

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The narrow host range of baculoviruses is one of their advantages for sustainable pest control of a single insect species with minimal or no effect on non-target organisms. However, the limited host range may be less desirable from the economical point of view since more than one pest can be present at the same time on most crops. Engineering baculovirus genomes with an expanded host range by design would be an answer to this type of scenarios. Nevertheless, genetic determinants of host range have not been widely characterized and the mechanisms of host recognition are still not well understood. In this context, the generation of hybrid or chimaeric baculoviruses may be an empirical approach to generate viruses with expanded host range. The expression of viral genes in this context will require the transcriptional transactivation of their promoters by heterologous transcription factors (TFs). However, the recognition of baculovirus promoters in different species has not been systematically studied so far. The aim of our work is to evaluate the transcriptional transactivation of betabaculovirus promoters by alphabaculovirus TFs. It has been noted before that late promoters require the replication of the DNA to be activated. Therefore, we generated stably transformed cell lines expressing the red fluorescent protein (DsRed) as a reporter gene under the control of immediate-early, early and late gene promoters of the Anticarsia gemmatalis nucleopolyhedrovirus (AgMNPV) and Epinotia aporema granulovirus (EpapGV), respectively. These cell lines were infected with AgMNPV to evaluate the transcriptional transactivation of these promoters. Our results showed that the AgMNPV transcription factors activate early and late EpapGV promoters.

Poster / Viruses. Wednesday, 16:30. **VI-16**

***Enhancin Genes of Lymantria dispar* NPV Do Not Increase Potency Via Metalloprotease Activity**

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The baculovirus encoded enhancins characterized so far are metalloproteases that digest proteins in the peritrophic matrix (PM) of their host midgut, increasing viral potency in many systems. *Lymantria dispar* NPV has two *enhancin* genes (E1 and E2) that are distributed in the ODV envelopes, placing them in a position to interact with the PM and the and possibly midgut cells. Deletion of either *enhancin* or both reduces viral potency 2-fold and 12-fold, respectively, compared to wildtype. Removal of the PM with optical brightener treatment did not alter these differences in potencies, suggesting that the enhancins do not affect the PM. The results of an *in vitro* PM digestion assay found that although the PM was degraded, it was not affected by inhibitors of metalloproteases, whereas treatment with serine protease inhibitors showed little or no PM degradation. Mutant LdNPV viruses were generated by altering the region that encodes the zinc binding site of the metalloprotease; this region of E1 and E2 was either deleted or altered by homologous amino

acid substitution to attempt to retain a functional enzyme. Bioassays showed that the deletion or alteration of just the zinc binding site of the metalloprotease, but not the entire enhancin gene, did not change viral potency. For example, a construct with E1 deleted/E2zinc modified had the same potency as E1deleted/E2 intact. These results suggest that the enhancins of LdNPV do not improve viral potency through the activity of metalloproteases, but appear to have a different mechanism, which has yet to be identified..

Poster / Viruses. Wednesday, 16:30. **VI-17**

A Cypovirus VP5 Displays the RNA Chaperone-like Activity that Destabilizes RNA Helices and Accelerates Strand Annealing

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For double-stranded RNA (dsRNA) viruses in the family Reoviridae, their inner capsids function as the machinery for viral RNA (vRNA) replication. Unlike other multishelled reoviruses, cypovirus has a single-layered capsid, thereby representing a simplified model for studying vRNA replication of reoviruses. VP5 is one of the three major cypovirus capsid proteins and functions as a clamp protein to stabilize cypovirus capsid. Here, we expressed VP5 from Helicoverpa armigera cypovirus-5 (HaCPV-5) in a eukaryotic system and determined that this VP5 possesses RNA chaperone-like activity, which destabilizes RNA helices and accelerates strand annealing independent of ATP. Our further characterization of VP5 revealed that its helix-destabilizing activity is RNA specific, lacks directionality, and could be inhibited by divalent ions, such as Mg2+, Mn2+, Ca2+ or Zn2+, to varying degrees. Furthermore, we found that HaCPV-5 VP5 facilitates the replication initiation of an alternative polymerase (i.e. reverse transcriptase) through a panhandle-structured RNA template, which mimics the 5'-3' cyclization of cypoviral positive-stranded RNA. Given that the replication of negative-stranded vRNA on the positive-stranded vRNA template necessitates the dissociation of the 5'-3' panhandle, the RNA chaperone activity of VP5 may play a direct role in the initiation of reoviral dsRNA synthesis..

Poster / Viruses. Wednesday, 16:30. **VI-18**

A recombinant *Autographa californica* nucleopolyhedrosis virus expressing a Cyt1A/GFP chimera in *Trichoplusia ni* larvae

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A novel approach was followed in order to achieve the expression of the Cyt1A toxin of *Bacillus thuringiensis* in a recombinant strain of AcNPV, trying to increase its virulence. First, the reporter GFP protein was used as a means to identify recombinant viruses; and second, biolistisc was used to achieve co-transfection. The recombinant construction pAccyta-GFP, containing the *B. thuringiensis* gene *cyt1A* fused with the GFP gene under the control of the p10 promoter from the pacuW31 vector was generated. Successful co-transfection by biobalistics was achieved with the AcNPV genome, when neonate *Trichoplusia ni* larvae were bombarded with DNA-coated gold micro-projectiles. Treated larvae showed the typical NPV infection symptoms, although only a thorough inspection detected fluorescent points in the fat body. Microscopic corroboration indicated that a recombinant AcNPV (AcNPV-cyt1a-GFP) was generated, showing glowing polyhedra in the infected cells, under fluorescence microscopy. This observation may indicate that the putative chimeric protein is incorporated into the polyhedron structure during its integration. Interestingly, the nuclei holding the recombinant polyhedra appeared less compact than those holding the wild-type polyhedra. A series of purification cycles of AcNPV-cyt1a-GFP rendered larvae showing fluorescence throughout the whole body. Preliminary observations indicate that AcNPV-cyt1a-GFP kills larvae faster than the wild-type strain; however, accurate LT_{50}s are still to be estimated.

Poster / Viruses. Wednesday, 16:30. **VI-19**

iLOV baculovirus: Using a novel small fluorescent protein for imaging virus proteins during infection

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Imaging of living cells is now a common place approach in cell biology and virus research, however addition of conventional fluorescent proteins such as green fluorescent protein (GFP) and its derivatives, can lead to alterations in the location and behaviour of target proteins. Such problems in mis-targeting have previously been observed on fusion of GFP to *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) P10, thus hindering imaging of P10 dynamics during virus infection of insect cells. Here we report use of iLOV, a small (~11kDa) genetically encoded fluorescent protein based on the LOV domain of plant phototropin 2, fused to P10 in AcMNPV. Expression of the P10-iLOV fusion during infection showed presence of filaments and nuclear structures, comparable to those seen in previous immunofluorescence images. We have also looked at the fluorescence lifetime of iLOV in P10 structures, where we established that the P10-iLOV fusion shows a very long fluorescence decay of ~4ns, compared to ~2.5ns for GFP. This work shows the successful use of iLOV in the baculovirus system, and provides an opportunity to tag proteins where GFP has previously failed. In addition the long fluorescence lifetime makes iLOV a promising candidate for use in protein interaction studies using Förster resonance energy transfer-fluorescence lifetime imaging (FRET-FLIM).

Poster / Viruses. Wednesday, 16:30. **VI-20**

Expression analysis of the *nsd-2* gene encoding the putative densovirus receptor in the midgut

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Bombyx mori densovirus type 2 (BmDNV-2) is a pathogen that replicates only in the midgut columnar cells and causes fatal disease in the silkworm. The resistance to BmDNV-2 is determined by a single gene, *nsd-2*, which is characterized as non-susceptibility irrespective of the viral dose. Previously we have identified *nsd-2* by positional cloning and found that this gene encodes a putative amino acid transporter which might work as a receptor for BmDNV-2. In this study, we investigated the relationship between the part of the midgut expressing *nsd-2* and the BmDNV-2 infection. To investigate the expression pattern of *nsd-2* in the midgut, we divided the midgut into three parts, anterior, middle, and posterior part, and performed the RT-PCR analysis with total RNA isolated from each part. *nsd-2* transcript

was strongly expressed in the posterior part of the midgut. However the expression levels of *nsd-2* were very low or no-detection in the anterior and middle parts. This regional expression pattern of *nsd-2* was common to all the investigated silkworm strain. On the other hand, the BmDNV-2-derived transcript was clearly detected in the posterior part of the midgut, but significantly lower in the anterior and middle parts. These results suggested that BmDNV-2 infection depended on the expression levels of *nsd-2* in the midgut. In insects, there is little information regarding the host's own factors in virus infection, therefore, we expect that our result will contribute to understanding the infection mechanism of insect virus. .

Poster / Viruses. Wednesday, 16:30. **VI-21**

Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*

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Viral covert infections in invertebrates have been traditionally attributed to sublethal infections that did not reach enough viral titer to establish an acute infection. Recent studies are revealing that, although true for some viruses, other viruses may follow the strategy of establishing covert or persistent infections without producing the death of the host. In the last years, a large number of viruses causing covert infections in all type of hosts have been identified, mostly due to the revolution in the sequencing technologies. The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) is a worldwide pest that causes significant loses to agricultural and ornamental plant industries. A comprehensive transcriptome analysis of the larval stage of *S. exigua* revealed the presence of an important number of unigenes belonging to novel RNA viruses, most of them from the order *Picornavirales*. In order to characterize *S. exigua* viral complex, we have completed the genomic sequences of three picorna-like viruses, two of them representing new members of the family *Iflaviridae* and a third one defining a new family. Additional studies have been performed to determine their morphology, infectivity, tissue distribution and abundance in the larval hosts. Influence of these viruses on the insect fitness as well as their effect on other viral and bacterial entomopathogens used for the control of this pest is also discussed.

Poster / Viruses. Wednesday, 16:30. **VI-22-STU**

A novel baculovirus-derived promoter with high activity in the Baculovirus Expression System

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In this work, we describe a novel baculovirus promoter for heterologous protein expression in insect cells using the baculovirus expression system. The promoter sequence is derived from the *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) genome and it was identified as potential promoter after transcriptional studies of the SeMNPV interaction with its host. First, an open reading frame (ORF) of SeMNPV was identified between the most highly abundant sequences in the transcriptome of *S. exigua* larvae infected with SeMNPV. Moreover, microarray-derived data showed high transcriptional activity of that ORF at different time points during the infective process. Different regions upstream of that ORF were tested for their promoter activity in the AcMNPV baculovirus expression system. Their ability to drive the expression of the GFP protein was compared against the polyhedrin (polh) conventional promoter in different cell lines, Sf21, Hi5, and Se301 and larvae from *S. exigua* and *Trichoplusia ni*. Although we found high levels of GFP expression with several regions, the strongest promoter activity was defined by 120 nt upstream the translation start site. GFP expression was up three times higher than the expression obtained with the polh promoter. Additionally, we also tested the activity for the combination of this sequence of 120 nt with the polh promoter revealing an additive effect over the polh promoter activity. This new promoter improves the conventional baculovirus expression system, allowing a considerable increase in the ability of producing large quantity of recombinant protein.

Poster / Viruses. Wednesday, 16:30. **VI-23**

Construction and Characterization of a Recombinant Invertebrate Iridovirus

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This study describes the construction and characterisation of a recombinant Chilo iridescent virus (family *Iridoviridae*) encoding the green fluorescent protein (GFP). We showed that homologous recombination is a valid method to make CIV gene knockouts and to insert foreign genes. The CIV 157L gene, putatively encoding a non-functional inhibitor of apoptosis (IAP), was chosen as target for foreign gene insertion. The *gfp* open reading frame preceded by the viral *mcp* promoter was inserted into the 157L locus by homologous recombination in *Anthonomus grandis* BRL-AG-3A cells. Recombinant virus (rCIV-Δ157L-*gfp*) was purified by successive rounds of plaque purification it was confirmed by PCR, sequencing and restriction analysis. One-step growth curves for recombinant and wild-type CIV were similar. Also slot blot analysis showed that DNA's of both recombinant and wild-type CIV started replication at the same time. Hence, CIV157L can be inactivated without altering the replication kinetics of the virus. Consequently, the CIV 157L locus can be used as a site for insertion of foreign DNA, e.g. to modify viral properties for insect biocontrol.

Poster / Viruses. Wednesday, 16:30. **VI-24**

RNA interference and insect-virus interactions

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Gene silencing via dsRNA has become a powerful tool to explore functional genomics in a wide variety of eukaryotic organisms.

However, RNA interference (RNAi) especially in Lepidoptera is not straight-forward and as efficient as in other insects and it is difficult to establish robust methods. So far, several potentially limiting factors for RNAi in Lepidoptera have only been proposed for *Bombyx mori*. An important role in the somewhat random success of RNAi in Lepidoptera could be the tissue-specific gene silencing effects, and also how the dsRNA is delivered to that tissue. To address this highly variable RNAi efficiency, we focused on the RNAi pathway (miRNA-pathway and siRNA-pathway) genes, and genes related to dsRNA transport or spreading in the Lepidopteran *Helicoverpa armigera* and *Heliothis virescens.* When analyzing RNAi-related gene expression levels in different larval tissues, we found that R2D2 is transcribed at very low levels in all tissues except testes, whereas Loquacious is transcribed at very high levels in all tissues. These results suggest that, despite appropriate design, dsRNAs could fail to enter the siRNA pathway, and to knock-down genes of interest due to the observed very low levels of R2D2. As the siRNA pathway is also known as the "antiviral pathway" and defends the organism against RNA and DNA viruses, we also aim at analyzing RNAi related genes (in vivo and in vitro) to an infection with *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Helicoverpa armigera* single nucleopolyhedrovirus (HaSNPV) - both as wild type and modified forms.

Poster / Viruses. Wednesday, 16:30. **VI-25**

Studies on existing and new isolates of *Cryptophlebia leucotreta* granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa

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Considering the possibility of some geographic populations of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) developing a reduced susceptibility to the baculovirus biopesticides, Cryptogran and Cryptex, as was the case with codling moth (CM), *Cydia pomonella* (L.) to the codling moth virus (CpGV) in Germany, the search for new isolates of the *T. leucotreta* baculovirus (CrleGV) become eminent. Here we report on the successful induction of a latent baculovirus infection in five geographic populations of *T. leucotreta* and the subsequent recovery of five new CrleGV isolates. These include the Ado, Cit, Mbl, Nels and MixC isolates. These isolates were shown to be genetically different from each other and from the commercial isolates, Cryptex and Cryptogran, using restriction enzyme analysis. The new isolates have been named CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl, CrleGV-SA Nels and CrleGV-SA Mix isolates. Sequence analysis of the *granulin* and *egt* genes of all isolates revealed single nucleotide polymorphisms (SNPs) in both genes. Significantly, SNPs in the *egt* genes of these isolates resulted in a change in amino acid sequence. DNA profiles from RFLPs, as well as phylogenetic analysis based on *granulin* and *egt* sequencing showed the presence of two CrleGV-SA genome types. Cryptex and CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl and CrleGV-SA Mix have been placed as members of Group one CrleGV-SA, and Cryptogran and CrleGV-SA Nels isolate placed into Group two CrleGV-SA. Studies on the comparative biological activity of the isolates also revealed significant differences between the relative potencies of the viral isolates against *T. leucotreta* from the Ado and MixC colonies..

Poster / Viruses. Wednesday, 16:30. **VI-26**

Effects of the baculovirus fibroblast growth factor on Sindbis virus replication

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Fibroblast growth factors (FGFs) are conserved among vertebrate and invertebrate organisms and function in cell proliferation, cell differentiation, tissue repair, and development. Many baculoviruses encode functional viral fibroblast growth factor (vFGF) homologs that stimulate cell motility of insect cells and activate host FGF receptors. During baculovirus infection of midgut lepidopteran cells, expression of vFGF leads to caspase activation and remodeling of tracheal epithelial cell basal lamina. Tracheal cell basal lamina remodeling results in structural discontinuities that allow baculovirus midgut escape. We hypothesized that vFGF would assist in midgut escape of the arbovirus Sindbis virus (SINV) during infection of mosquitoes. We first verified that vFGF stimulated cell motility in two mosquito cell lines, C6/36 and Aag2. Utilizing an alphavirus transducing system for SINV, we then constructed recombinant SINVs expressing *vfgf* (MRE/vFGF, TE/vFGF), and control viruses with the same insert in antisense orientation (MRE/vFGFas, TE/vFGFas). TE-based viruses replicate in cell cultures but poorly infect mosquito midguts, while MRE-based viruses infect midguts efficiently. Replication of each vFGF-expressing virus and its control virus was similar in both cell lines. Female *Aedes aegypti* mosquitoes orally infected with each of the recombinant viruses had no significant replication differences, measured by determining infectious viruses in individual mosquitoes, mosquito midguts, or carcasses. Thus, it does not appear that expressing vFGF affects SINV replication and dissemination.

Poster / Viruses. Wednesday, 16:30. **VI-27**

Sensitivity and vertical transmission of nuclearpolyhedrovirus in various populations of gypsy moth *Lymantria dispar*

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The gypsy moth is known as the most biologically and economically important pest species. Nucleopolyhedrovirus (LdNPV) is one of the key factors that influence on the gypsy moth population density. The different sensitivity of larvae gypsy moth LdNPV from different nature population was registered. This sensitivity of insects may depend on percentage of occult virus in insects populations. High susceptibility of larvae to virus was registered in population with high level (91 ± 7%) occult virus as compared to population with lower level (48 ±5%) of occult virus. In addition for detection of virus transmission during several generations the larvae of parents generation were infected with high (modeling of epizootic) or low doses (modeling of sporadic death) of the LdNPV. Enhanced insects mortality caused by spontaneous virus infection in three progeny generations has been shown for parents infected by both doses of virus compared to non infected control. The level of occult virus was in 2-fold decreased to third generation for all cases. However occult virus has been detected up to sixth generations just in case of parents'

infected with high dose of virus. Possibly exogenous insect virus may be activator of viral infection and lead to epizootic. However sometimes exogenous virus produces transgenerational occult form.

Poster / Viruses. Wednesday, 16:30. **VI-28**

Establishment of SeMNPV Persistent Infection and Screening of Persistent Infection Associated Genes in Baculovirus

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Persistent baculovirus infection is observed in insect populations. Persistent infection can be transformed to a replicative and infective state and plays an important role in epizoology of baculoviruses. However, the molecular mechanism of baculoviral persistence is unknown. *Spodoptera exigua multiple nucleopolyhedrovirus* (SeMNPV) was serially undiluted passaged in Se301 cells to reduce virulence. Upon infection of Se301 cells with the SeMNPV up to passage 8, a few cells survived even if most of cells died due to virus infection. The surviving cells were passaged and designated as P8-Se301 cells. The cells continually released infectious progeny virus and show a typical character trait of baculovirus persistent infection. Using limited dilution method, a cell clone was isolated and designated as P8-Se301-C1. The cells were morphology similarly to the Se301 cells, and no polyhedra or viral particles were observed. However, incomplete SeMNPV genomes and low level SeMNPV transcripts presented in P8-Se301-C1 cells. It was suggested that a latent-like viral infection is present in the P8-Se301-C1 cells. To screen and identity the persistent infection associated genes in baculovirus, The total protein was extracted and isolated through 2-D gel electrophoresis, the differential expression were analyzed between the P8-Se301-C1 cells and the healthy Se301 cells. It would provide a basis for further exploring the molecular mechanisms of baculoviral persistence. .

Poster / Viruses. Wednesday, 16:30. **VI-29-STU**

Larvicidal activity of an ascovirus from *Spodoptera litura* against parasitoid wasps

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When the endoparasitoid *Cotesia kariyai* (Hymenoptera: Braconidae) parasitizes *Mythimna separata* larvae infected with *M. separata* entomopoxvirus (MySEV), the parasitoid larvae die in the infected host. Death is caused by a 28-kDa polypeptide, named parasitoid killer toxin (PKT), which is encoded by the MySEV genome and secreted from MySEV-infected fat body cells into the hemolymph of an infected larva. *pkt* gene homologues are found not only in entomopoxviruses but also in other insect viruses including granuloviruses, nucleopolyhedroviruses and ascoviruses (AVs). AVs are double-stranded DNA viruses and mainly infect noctuid larvae, producing symptoms that include stunted growth and opaque white hemolymph. A unique characteristic of AVs is their poor *per os* infectivity; in nature, AVs are transmitted by the ovipositors of female parasitoid wasps. Since AV transmission thus coincides with wasp oviposition, parasitoid wasp larval mortality in an AV-infected host has sometimes been attributed to the AV, although no known mechanism explains such larvicidal activity. To elucidate whether the *pkt* homologue in AVs is involved with this larvicidal phenomenon, we sequenced *pkt* homologue in an AV isolated from *Spodoptera litura* in Japan, and found that its predicted amino acid sequence displayed identity with MySEV PKT in a 750-bp partial sequence. Hemolymph from AV-infected larvae showed larvicidal activity against *C. kariyai* and *Microplitis* sp. (Braconidae) larvae. These results suggest that PKT expressed from the AV genome can cause death of braconid parasitoid larvae in hosts infected with the AV isolate.

Poster / Viruses. Wednesday, 16:30. **VI-30**

"11K" genes family *sf68*, *sf95* and *sf138* modulate transmissibility and insecticidal properties of *Spodoptera frugiperda* multiple nucleopolyhedrovirus

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The "11K" protein family is notable for having homologs in both baculoviruses and entomopoxviruses. These genes are classified as either type 145 or type 150, according to their similarity with *ac145* or *ac150* of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). One homolog to *ac145* (*sf138*) and two homologs to *ac150* (*sf68* and *sf95*) are present in *Spodoptera frugiperda* multiple nucleopolyhedro-virus (SfMNPV). Recombinant viruses lacking *sf68*, *sf95* or *sf138* (Sf68null, Sf95null and Sf138null, respectively), and the respective repair viruses, were generated from a bacmid comprising the complete virus genome. Occlusion bodies (OBs) of the Sf138null virus were ~15-fold less pathogenic to insects, which was attributed to a 100-fold reduction in ODV infectious titer/OB. Inoculation of insects with Sf138null OBs in mixtures with an optical brightener failed to restore the pathogenicity of Sf138null OBs to that of the parental virus, indicating that the effects of *sf138* deletion on OB pathogenicity were unlikely to involve an interaction with the gut peritrophic matrix. In contrast, deletion of *sf68* and *sf95* resulted in a slower speed-of-kill by ~7%, and a concurrent increase in the total production of OBs/larva. Phylogenetic analysis indicated that *sf68* and *sf95* were not generated after a duplication event of the *ac150* gene. We conclude that type 145 genes modulate primary infection process of the virus, whereas type 150 genes appear to have a role in spreading systemic infection within the insect.

Poster / Viruses. Wednesday, 16:30. **VI-31**

Characterization of two ORFs undergoing positive selection in a genotype of *Chrysodeixis chalcites* single nucleopolyhedrovirus from the Canary Islands

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The availability of genome sequences of different genotypes from a single isolate could help explain phenotype differences related to changes at genome level. Here we report the complete genome sequence of five genotypes of a *Chrysodeixis chalcites* single NPV isolate from the Canary Islands (named ChchSNPV-TF1-A, -B, -C, -G and -H). The whole genome sequences of the

ChchSNPV-TF1 genotypes are 99% identical to the previously reported ChchSNPV strain from The Netherlands (ChchSNPV-NL). ChchSNPV-TF1-A, -B, -C, -H genomes did not present ORF 53 of unknown function that is unique to ChchSNPV genomes. Major regions of variability among ChchSNPV genomes was identified in the *hoar* and *bro-d* genes. In an effort to identify genes potentially involved in virulence or in determining population level adaptations, selection pressure analysis was performed. Five ORFs were identified as undergoing positive selection; *chch55* (*bro-a*), *chch65* (*chitinase*), *chch69* (*bro-b*), *chch143* and *chch144*, the last two of which are of unknown function. Strong selection for *bro* and *chitinase* genes indicates that viral replication and liquefaction processes are critical points at which adaptation acts during transmission of these viruses. Among the unknown ORFs, *chch143* exhibits a high degree of similarity with the metalloprotease superfamily and with the previously characterized *sf29* of *Spodoptera frugiperda* multiple nucleopolyhedrovirus involved in ODV packaging. Experiments are in progress to determine the function of *chch143* and *chch144* in the transmission of ChchSNPV.

Poster / Viruses. Wednesday, 16:30. **VI-32**

Genome sequence and organization of a *Betabaculovirus* pathogenicto cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae)

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The cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae), is a very severe pest in cassava (*Manihot esculenta*) due to its worldly geographical distribution and high capacity of leaf consumption. It is also the most serious pest of the rubber tree (*Hevea brasiliensis*) in Latin America. The *Baculovirus Erinnyis* has been shown to be an economically viable and safe biopesticide for controlling this pest in South America. In the present work the complete sequence of the *Erinnyis ello granulovirus* (ErelGV) genome was determined. The viral DNA was extracted from a viral isolate collected in South Brazil, in 1986. Analysis by transmission electron microscopy showed granular occlusion bodies with single virions inside the protein matrix, confirming that this pathogen is a *Betabaculovirus*. The genome is 102,759 bp with G+C content of 38.7%, being larger than the previous estimation of 90,000 ± 5,000 bp based on restriction mapping for a Colombian isolate. A total of 130 putative ORFs were found encoding at least 50 amino acids. Eight of these were shown to be unique (*ErelOrf-11*, *ErelOrf-15*, *ErelOrf-27*, *ErelOrf-53*, *ErelOrf-59*, *ErelOrf-70*, *ErelOrf-90*, *ErelOrf-102*), and all the predicted protein had no significant similarity to any other sequences in GenBank. ErelGV is closely related to *Choristoneura occidentalis granulovirus* (ChocGV) and *Pieris rapae granulovirus* (PiraGV). No typical homologous regions (hrs), *cathepsin* or *chitinase* genes were detected. *Alphabaculovirus* horizontal gene transfer, such as *he65* and *p43* homologous genes, was found. Moreover, a nucleotide metabolism-related gene and two genes acquired probably from *Densovirus* were also detected.

Poster / Viruses. Wednesday, 16:30. **VI-33-STU**

Analysis of genetic interactions among four non-essential genes of BmNPV

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Nucleopolyhedroviruses (NPV) produce copious amounts of polyhedrin (Polh) by the end of a replication cycle and form a lot of polyhedra in the nuclei of infected-host insect cells. This characteristic feature of NPV is a beneficial trait as the gene expression vector. To further develop baculoviral applications, deep insight into the functions and interactions of viral gene products concerning the explosive expression of Polh is necessary.We constructed a library of single gene knockout BmNPVs and showed that 86 out of 141 viral genes were dispensable for expression of the polyhedrin gene and production of infectious viral progenies (Ono et al., 2012). However, it has not been examined how these non-essential genes in combinations contribute to the viral infection. We then started a study to understand the genetic interactions among the non-essential genes. In this present study, we constructed BmNPVs lacking multiple non-essential genes and analyzed the expression of EGFP under the control of the polyhedrin gene promoter. Synergistic, compensatory, and additive relationships were observed in the genetic interaction analysis between pairs of adjacent genes in the *orf11-12-13-14* gene cluster. The results in this study revealed complex genetic interactions among the non-essential genes of BmNPV.

Poster / Viruses. Wednesday, 16:30. **VI-34 STU**

Comparative fitness of a granulovirus mutant possessing larger occlusion bodies than wild type *Adoxophyes orana* granulovirus

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During host infection, the virus particles of baculoviruses become embedded within a large proteinaceous occlusion body (OB). Outside the host, the OB protects virus particles from environmental factors including ultraviolet light (UV). However, the mechanism(s) that determine the morphology (size and shape) of OBs are not understood. We isolated a novel mutant of *Adoxophyes orana* granulovirus (AdorGV) from an *A. honmai* larva in a tea field in Japan. This mutant AdorGV produced cube-shaped OBs with edges of approximately 1.0 µm, whereas a wild type (WT) AdorGV isolated in the UK produces typical ellipsoidal OBs of approximately 0.5 µm in length. According to its full genome sequence, the mutant AdorGV was closely related to WT AdorGV. Since such giant OBs should be more costly for the virus to produce than the smaller WT AdorGV OBs, the mutant AdorGV may exhibit a trade-off in production fitness to acquire other adaptive traits. In this study, the UV tolerance of mutant AdorGV was compared to that of WT AdorGV. The persistence of the mutant AdorGV was four times longer than that of the WT AdorGV. The UV tolerance of OB-derived virus particles of mutant and WT AdorGV showed no significant difference. Thus, we elucidate that mutant AdorGV have high UV tolerance to produce giant OBs. This trait may be trade-off of some cost of mutant AdorGV such as production cost of giant OBs.

Poster / Viruses. Wednesday, 16:30. **VI-35**

Granulovirus detection in larvae of sugarcane borers *Diatraea* spp. (Lepidoptera: Pyralidae) in Colombia

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Panela is a solid piece of unrefined sucrose obtained from evaporation of sugarcane juice, a very important industry and source of employment in Colombia. Panela yield depends of sucrose content in sugarcane, characteristic seriously affected by the presence of the stem borers complex, difficultly controlled by chemical insecticides, being an alternative the use of biological agents as granuloviruses. In this sense, the objective of this work was to isolate granulovirus naturally infecting *Diatraea* spp. larvae in sugarcane crops for panela production in Colombia. Larvae were collected from three different production areas and maintained in quarantine until dead. A total of 445 larvae were collected, 227 in Boyacá, 130 in Santander and 88 in Nariño. From collected larvae, 39 individuals died showing disease symptoms. Five dead larvae showed fungal mycelium growth and 34 presented sings of viral infection, which were analyzed by granulin gen QPCR, complete granulin gen PCR with degenerated primers and Dot Blot by using polyclonal antibodies for granulin produced in hen eggs. Two samples from Boyacá and two samples from Santander were positive by molecular and immunological methods, being three detected by QPCR and Dot Blot simultaneously and one from Boyacá positive by the three evaluated techniques. Only the 0.89% of collected larvae evidenced viral infection by granulovirus, which were detected by using very low volumes of crude samples. All methods showed to be promising for detecting granulovirus in field samples and four detected virus will be amplified in the insect for a further characterization and biopesticide development.

Poster / Viruses. Wednesday, 16:30. **VI-36**

Earthworm-mediated dispersal of baculovirus occlusion bodies in soil: a laboratory study

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Soil is an important environmental reservoir of baculovirus occlusion bodies (OBs). The multiple nucleopolyhedrovirus (SfMNPV, genus *Alphabaculovirus*) of *Spodoptera frugiperda* has attracted attention as a potential biological insecticide for control of this pest in maize and sorghum crops in the Americas. This study examined the potential role of the earthworm *Eisenia fetida* as a possible disperser of SfMNPV OBs in a model laboratory system. A soil incorporation bioassay technique was calibrated using *S. frugiperda* second instars that fed on an OECD artificial soil (70% sand, 20% kaolin, 10% peat) contaminated with SfMNPV OBs ($5x10^4$ - $5x10^9$ OBs/ml). The LC_{50} value was estimated at 2.3×10^8 OBs/ml. The gut pH of *E. fetida* was estimated to be pH 5.0-6.0 using pH indicators. Earthworms burrowed 22.5 cm into experimental soil in a 72 h period. Earthworms redistributed SfMNPV OBs vertically by up to 22 cm in artificial soil over periods of 1, 7 and 15 days. Incubation of earthworms in OB treated soil for 7 days did not significantly affect the insecticidal activity of the OBs compared to OBs in soil in the absence of earthworms ($P>0.05$). This represents a previously unrecognized mechanism of baculovirus dispersal in the environment that is likely to have important implications in the persistence of OB populations in soil reservoirs.

Poster / Viruses. Wednesday, 16:30. **VI-37-STU**

Effects of rearing temperature on the susceptibility of larvae of the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae) to *A. honmai* nucleopolyhedrovirus

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Many environmental factors, such as ultraviolet, humidity and temperature, can affect the susceptibility of insect hosts to entomopathogens. Among these factors, temperature is one of the most important factors for both insect susceptibility and multiplication of entomopathgens in the host. The smaller tea tortrix, *Adoxophyes honmai*, is one of the most important pests of tea plants in Japan and occurs four or five times in a year. In addition, larvae of *A. honmai* live in a wide range of temperature from 0°C to 35°C. Here, we examined the effects of high temperature on the susceptibility of *A. honmai* larvae to *A. honmai* nucleopolyhedrovirus (AdhoNPV). Fifth instar larvae of *A. honmai* were exposed to AdhoNPV by the modified droplet feeding method and reared on artificial diet at 25°C, 28°C, 31°C or 34°C. The susceptibility of *A. honmai* larvae was reduced with an increase in rearing temperature. No AdhoNPV-infected larvae were observed when larvae were reared at 34°C. The infection rates of *A. honmai* fifth instar larvae that were reared at 34°C were significantly lower than those of larvae that were reared at 25°C, 28°C and 31°C when budded viruses of AdhoNPV were injected.

Poster / Viruses. Wednesday, 16:30. **VI-38**

Characterization of Nodaviral Protein A Revealed RNA Synthesis and Terminal Nucleotidyl Transferase Activity

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Nodaviruses are a family of positive-stranded RNA viruses with a bipartite genome of RNAs, RNA1 and RNA2. Protein A, which is recognized as an RNA-dependent RNA polymerase (RdRP), is encoded by genomic RNA1 and functions as the sole viral replicase protein responsible for its RNA replication. Although nodaviral RNA replication has been studied in considerable detail, the mechanism(s) governing the initiation of nodaviral RNA synthesis have not been determined. In this study, we characterized the RdRP activity of Wuhan nodavirus (WhNV) protein A and Flock House virus(FHV) in detail and determined that these nodaviral protein A initiates RNA synthesis via a de novo mechanism. Moreover, we uncovered that both of WhNV protein A and FHV protein A possess terminal nucleotidyl transferase (TNTase) activity. We subsequently found that the TNTase activity of WhNV protein A and FHV protein A could function in vitro to repair the 3' initiation site, and may function as a rescue and protection mechanism to protect the 3' initiation site, and ensure the efficiency and accuracy of viral RNA synthesis. Furthermore, we determined the cis-acting elements for RdRP or TNTase activity at the 3' end of positive- or negative-strand RNA1. Altogether, our study establishes the de novo initiation mechanism of RdRP and the terminal rescue mechanism of TNTase for WhNV and FHV protein A, and represents an important advance toward understanding nodaviral RNA replication.

Non-Target Effects on Biological Pesicides Transgenic Crops

Workshop paper. Wednesday, 20:00 **199**

The impact of herbicide tolerant crops on non-target organisms

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Tolerance to broad spectrum herbicides is the most worldwide cultivated transgenic trait and millions of hectares have been sown with herbicide tolerant (HT) soybeans, maize, and canola. Among potential effects of this kind of genetically modified (GM) crops on the environment are those on non-target organisms (NTOs). A NTO is any species that is not the direct target of the GM crop and may include non-target plants (particularly in the margins and nearby habitats), plant pathogens, arthropods birds and wildlife, and a diversity of soil organisms. The impact of HT crops on non-target organisms may be exerted through three main mechanisms: (i) the direct effect of the trait introduced into the plant on the NTO, (ii) the effect of the herbicide on the NTO, and (iii) through the food web. While there are no records in the literature of any effect through the first mechanism to our knowledge, and relatively very few through the second one, more effects have been described through trophic relationships mainly originated by the alteration of the abundance, composition and phenology of weed flora. This presentation is mainly focused on this third mechanism and particularly on weed- arthropods relationships as the first trophic interaction that leads to build complex food webs in agroecosystems. According to the experience of Spanish field trials with HT maize, few changes in NTO populations may be expected if modifications of weed flora are not dramatic. Potential benefits derived from the flexibility of timing broad spectrum herbicide sprayings are discussed.

Workshop paper. Wednesday, 20:15 **200**

Your Right to Know What You Eat: On the Occurrence of Viable*Bacillus thuringiensis* in Commercial Food Products

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It is widely recognized in the scientific community that genetically engineered crops are safe for human consumption. Yet serious concerns continue about the safety of these foods, despite consumption of approximately two trillion meals by people over the past decade with no known ill effects. During 2013, for example, new laws were proposed in California and Washington State to label foods containing genetically modified organisms (GMOs), the rationale being that people have a right to know what they eat. Although both laws failed, there is little doubt the public remains concerned about GMO food safety. Unknown to the public and many scientists is that *Bacillus thuringiensis* (Bt), the source of the insecticidal proteins used in insect-tolerant crops such as Bt corn and Bt soybeans, occurs naturally and commonly on many vegetables, grains, and nuts, including products based on these such as flour and flour products (bread, pasta), cereals, soup, salami, candy and puddings. Moreover, and ironically, the only insecticides permitted for use on organic crops are Bts that contain viable spores and the same Cry proteins used in GMO crops. Whether due to natural occurrence or the use of Bt insecticides, these foods can contain hundreds to thousands of viable spore/crystal mixtures per gram or cm^2. In this presentation, I will review the data showing that Bt occurs naturally and commonly in our food supply, and that the diversity of strains and insecticidal proteins which people consume is much greater than those used in commercial Bt insecticides or GMO crops.

Workshop paper. Wednesday, 20:30 **201**

Environmental risk assessment of genetically engineered crops for spiders

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Before genetically engineered (GE) crops can be grown commercially, potential risks to the environment need to be assessed. Environmental risk assessment (ERA) ensures that desired ecological functions (protection goals), such as biological control, pollination, and decomposition are not harmed. We will present the process of non-target ERA for GE plants producing insecticidal proteins derived from *Bacillus thuringiensis* (Bt). Spiders are among the most abundant biological control agents in arable systems and we will use examples from our research to illustrate the different ERA steps.
The populations of species associated to the ecosystem services to be protected represent assessment endpoints for the ERA. Knowledge on the community inhabiting the GE crop grown in a certain region (receiving environment) is combined with knowledge on potential exposure and sensitivity to the insecticidal compound to focus the assessment and to formulate relevant risk hypothesis to be tested. The different risk hypotheses are then addressed in the analysis phase of the ERA following a tiered approach. Early-tier testing is conducted under worst-case exposure conditions in the laboratory. Surrogate test species are selected that are most likely to reveal an adverse effect. More complex and realistic semi-field or field studies supplement the ERA when uncertainty about the level of risk to non-target species remains high after early tier laboratory studies are conducted. We will discuss important criteria to consider when designing non-target studies, which can only inform the ERA if they are reproducible, reliable, and test clearly defined risk hypotheses.

Workshop paper. Wednesday, 20:45 **202**

Conclusions from 10 years of accumulated evidence from publicly funded field trials research with Bt-maize in Germany

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Publicly funded research into the environmental risks of genetically modified plants has been performed in Germany for more than a decade. The Bt-maize events MON810, MON88017 and MON89034 x MON88017 were assessed in field trials. Each of the trials lasted for 3 years and lead to the further refinement of trial designs and assessment methods. The combined results on non-target organism effects show that a) the assessed Bt-maize events do not harm the communities of NTOs typical for maize; b) conventional treatments with

insecticides can have profound negative impacts; c) conventionally bred maize varieties can differ substantially in their impact; d) different management practices have profound impacts on populations on-crop and off-crop. A number of conclusion can be drawn from the assessments: 1. The NTO ERA for Bt-maize should more strongly rely on early tier experiments; 2. Field trials are only sensible if results from earlier tiers show the possibility for negative NTO impacts; 3. A comparative approach to ERA is without alternative, also looking at conventionally bred varieties and alternative management approaches; 4. The methods and trial designs used are able to detect differences in impact of different maize varieties; 5. To fully assess the potential impacts of the cultivation of Bt- and other genetically modified plants a systems approach is needed, that also takes into account the benefits of using these plants; 6. A decision is needed on what we really want to protect and thus need to assess.

THURSDAY - 7 August

SYMPOSIUM 7 (Dis. of Benef.l Inverteb.) Thursday, 8:00-10:00

Emerging Tools for Aquatic Pathogen Discovery and Description

Symposium. Thursday, 8:00. **203**

Early mortality syndrome is an infectious disease with a bacterial etiology

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Beginning in about 2009, a new, emerging disease called "Early Mortality Syndrome or EMS" (more descriptively called Acute Hepatopancreas Necrosis Syndrome or AHPNS) began to cause significant production losses in shrimp farms southern China. By 2010 the range of affected farms in China had expanded, and by 2011 EMS/AHPNS was confirmed in Vietnam and Malaysia, and in Thailand in 2012. EMS/AHPNS disease has caused serious losses in the areas affected by the disease, and it has also caused secondary impacts on employment, social welfare, and international market presence. EMS/AHPNS was first classified as an idiopathic disease because no causative agent had been identified. Preliminary studies conducted in Vietnam in 2012 by the Laboratory of Aquaculture Pathology at the University of Arizona (UAZ-APL) have indicated that EMS/AHPNS is infectious. Since early in 2013, the UAZ-APL was able to isolate and identify the causative agent of EMS/AHPNS in pure culture. In several separate challenge experiments, the same EMS/AHPNS pathology was reproduced consistently in experimental shrimp. In addition, the same identical agent was recovered from the challenged animals and several subsequence challenge tests using the recovered agent could also reproduce EMS/AHPNS pathology with very consistent results. The agent was identified as a unique strain of *Vibrio parahaemolyticus* that is commonly found in marine environment. Hence, EMS/AHPNS has a bacterial causative agent that satisfies Koch's Postulates to be a typical infectious disease. Further studies focusing on the agent of AHPNS revealed that the agent could produce toxin(s) causing the primary pathology in affected shrimp.

Symposium. Thursday, 8:30. **204**

Policy, phylogeny, and the parasite

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Animal diseases gain political attention by their inclusion on lists of global bodies such as those of the World Organisation for Animal Health. Currently, the OIE lists 116 diseases caused by viral, bacterial, fungal, protistan, and metazoan pathogens. Each is afforded a specific chapter in the regularly updated OIE 'Manual of Diagnostic Tests' series. Of these, 30 diseases are caused by eukaryotic (fungal, oomycete, protistan, and metazoan) parasites. Inclusion necessitates national governments to report outbreaks promptly but may lead to trading restrictions between nations in an attempt to limit spread. Detection therefore has consequences that may directly impact from farm to state levels. Here, we consider current approaches to discrimination of listed parasites from related, but unlisted, counterparts. We outline problems with defining 'species', propose the necessary drivers that should be required for discrimination of important taxa, and highlight how this process may be influenced by national policies. Further, we propose a set of 'best practice' measures, broadly based upon current taxonomic philosophies for protists and metazoans that should be applied when defining taxa for listing as notifiable. We will illustrate these principles with topical issues associated with the taxonomy and listing of aquatic invertebrate pathogens.

Symposium. Thursday, 9:00. **205**

The Next Generation of Crustacean Health: Disease Diagnostics Using Modern Transcriptomics

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Commercial crustacean fisheries on the Atlantic coast of Canada represent over $(CAD) 1 billion annually. The American lobster (*Homarus americanus*) fishery alone represents over $(CAD) 600 million with harvests in recent years breaking records for amount of lobster that has been landed. The Canadian and Maine USA lobster populations remain healthy but the once vibrant lobster fisheries in Southern New England USA have been devastated by a mixture of disease, ocean acidification, global warming and anthropogenic stressors. Conventional gross anatomic, microscopic and histological analysis remain the backbone of

crustacean health and disease assessment but new molecular genetic techniques are beginning to be integrated into this assessment. Modern genomics and transcriptomics have revolutionized the discovery of diagnostic and prognostic markers in human and terrestrial medicine and promise to drive crustacean health and diagnostic molecule discovery. We have recently begun to apply high-throughput transcriptomic techniques, such as microarray and RNA-Seq, to investigate American lobster health, disease and response to physiological and anthropogenic stressors. Our studies highlight the incredible potential that modern molecular biological approaches have for advancing our understanding of crustacean immunology and disease biomarker discovery.

Symposium. Thursday, 9:30. **206**

Environmental DNA as a tool for detection and identification of aquatic parasites: known unknowns and just plain unknowns

Hanna Hartikainen[1,5]; Grant D. Stentiford[2,3]; Kelly Bateman[2,3]; Stephen W. Feist[3]; David M. Stone[3]; Matt Longshaw[3,4]; Georgia Ward[1]; Charlotte Wood[1]; Beth Okamura[1] and David Bass[1]

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The increasing application of massively parallel sequencing technology to environmental DNA samples (e.g. from water, sediment, soils, whole animals) is providing unprecedented resolution of microbial community structure, diversity and functioning. Application of general and specific primer approaches, amplicon sequencing and metagenomics have enormous potential for the detection of known, novel and otherwise cryptic pathogen lineages. We use such techniques to detect invertebrate pathogens of potential significance to fisheries and aquaculture. Using specific-primer approaches, we have revealed unknown diversity of haplosporidian parasites from eDNA and show shifts in parasite communities along an offshore gradient. At the other end of the spectrum, we have used a metagenomic approach to identify a mikrocytid pathogen of juvenile edible crabs that had eluded molecular characterization using specific- and general primer approaches. We highlight the current methods for discovery and detection of potential pathogens in eDNA samples and show how such studies can inform on ecology, life-cycle and transmission dynamics of aquatic pathogens. Finally, we predict a re-emergence in the importance of classical approaches to disease investigation (e.g. histopathology, electron microscopy) to enable meaningful links to be drawn between presence within the matrix and outcomes in hosts. eDNA analyses should therefore be considered as a 'tool in the box', rather than the toolbox per se, for investigating pathogens of concern to aquatic hosts.

Nematodes 3

Contributed paper. Thursday, 8:00. **207**

The Role of biocontrol agents within IPM of *Tuta absoluta* on tomato in Egypt

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Since its arrival in Spain, the tomato leafminer *Tuta absoluta* has rapidly spread around Europe and has become an extremely important pest of tomato crops in Mediterranean Basin countries. This pest arrived to Egypt early in 2010 and there soon followed an outbreak in many tomato-planted areas where it caused extensive damage by mining in tomato leaves, stems and fruit. Egyptian entomopathogenic nematode species (EPN) induced 89.3-96.4% mortality to *T. absoluta* larvae. Also, the other biocontrol agents *Trichogramma achaeae* and *Macrolophus pygmaeus* are suggested as effective components within a new control strategy against the insect on tomato in the present study. *M. pygmaeus* may prey on *T. absoluta* eggs and larval stages, but due to more suitable climate of Egypt to *T. achaeae*, earlier release of the latter bug is preferable in order to start the control on the first generations of the pest eggs. EPN have both foliar and soil applications in the strategy. On the foliage, EPN can control efficiently feeding larvae of *T. absoluta* in and outside the leaf galleries while the soil nematodes kill both last instar larvae, when they slide down from the leaves to pupate, and emerging adults from the buried pupae. In addition to such natural enemies, the strategy is supported by prophylactic measures, light and pheromone traps, and IPM compatible insecticides.

Contributed paper. Thursday, 8:15. **208**

Insecticidal activity of *Heterorhabditis bacteriophora* Shandong toward *Brontispa longissima* and *Cryptothelea variegate*

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Heterorhabditis bacteriophora nematodes kill many insect species, but its potencies toward *Brontispa longissim and Cryptothelea variegate* pests are unknown. Initially, four isolates of *H. bacteriophora*, UV resistant *H. bacteriophora* Shandong (HbSD), Hb I, Hb II, and Hb III were bioassyed against standard insect *Galleria mellonella*. The UV resistant HbSD isolate was chosen for next bioassay against the last-instar of *B. longissima* and *C. variegate* compared with *G. mellonella* in the laboratory. After exposure of insects to infective juveniles of nematodes (IJs) for six days, mortality was correlated with dosage, and the LC_{50} was ≈ 9.35 IJs for *B. longissima* and ≈ 11.76 IJs for *C. variegate*, as compared with that ≈ 8.56 IJs for *G. mellonella*. There are no statistically different in potency among these three hosts. Thus, the insecticidal potencies of the nematodes to these three pests

were: *C. variegate* = *B. longissima* = *G. mellonella*. However, there is a significant dose-response in each treatment of the insect species. Two field trials were conducted in local residence yards in the Wanning City suburb of Hainan province, P. R. China. The results showed that after spraying *H. bacteriophora* SD IJs in the period of March and April, *Cinnamomum camphora* trees is significant difference in the survival rate between the treatment and untreated control ($p < 0.05$). The technology presented may be of substantial interest to biological pesticide producers.

Contributed paper. Thursday, 8:30. **209**

Prospects for using Entomopathogenic Nematodes to Control the Vine Mealybug, *Planococcus ficus*, in South African Vineyards

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Planococcus ficus (Signoret), the vine mealybug, is regarded a major pest insect of the South African grape industry. Mealybugs are difficult to control with chemicals due to their cryptic lifestyle of hiding in crevices, under bark and below ground on roots where chemicals battle to reach. Another problem in the use of chemical pesticides is the water repellent waxy secretions and the ability of mealybugs to rapidly build up resistance. Entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae can potentially be used within an integrated pest management system to control the vine mealybug, which not only occur mostly on the aerial part of plants, but also on the roots. Both local *Heterorhabditid zealandica* and *Steinernema yirgalemense* were able to move 15 cm downward in sand columns to infect *P. ficus*, with respective mortalities of 82% and 95%. Laboratory persistence of *S. yirgalemense* in sterile, moist sand in the laboratory remained high (> 85%) after 6 months, while that of *H. zealandica* dropped to 5%. When *S. yirgalemense* was applied to the soil of two vineyards with adult female *P. ficus*, contained in pierced Eppendorf tubes, buried at a depth of 15 cm in the soil, mortalities of up to 50% were obtained after 48 h. Persistence of *S. yirgalemense,* measured using codling moth larval mortality, was found to be zero in one vineyard, while in the other 70%, 12 weeks after application. These studies showed that entomopathogenic nematodes, specifically *S. yirgalemense*, have promising potential as biological control agents for *P. ficus* soil populations.

Contributed paper. Thursday, 8:45. **210**

New data on *Steinernema ichnusae* distribution in the Mediterranean Area

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The species *Steinernema ichnusae* (Tarasco, Mráček, Nguyen & Triggiani, 2008) have been isolated till now only from Sardinia (Tarasco *et al*., 2014; doi: 10.1017/S0022149X14000194). Recent molecular studies carried out on some strains isolated in other Mediterranean areas revealed this species is also present out of Sardinia island. Five strains of *S. ichnusae* were identified coming from different coastal sites in Algeria (ALG2, ALG3, ALG15, ALG 16 and ALG18), one from continental Italy (Campania Region, MU1) and two from Sicily (EMA 2 and CT026). All these strains had previously been only partially identified as belonging to a species of the *S. feltiae* group. The molecular studies showed that all the strains examined shared with *S. ichnusae* some nucleotide changes in the ITS1 region, including a very conserved 10 bp composite deletion. This makes it easy to setup a molecular assay to discriminate *S. ichnusae* from the close species *S. feltiae*. These new results show that this species is not endemic of Sardinia, as previously believed, and it might be widespread in other Mediterranean Countries as well.

Contributed paper. Thursday, 9:00. **211-STU**

Evaluation of entomopathogenic nematodes for control of the diapausing overwintering codling moth population

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In South Africa codling moth (CM) (*Cydia pomonella*) is the most important insect pest of apples and pears. During the winter months, from April to August, no fruit is on the trees, and the total CM population overwinters as diapausing larvae. During this period, entomopathogenic nematodes (EPNs) can be applied to reduce the number of emerging moths in the following season. The impact of aerial EPN application, and environmental conditions, on CM larvae mortality was investigated in an apple orchard. CM larvae were used to culture infective juveniles, used in the different field trials. As containment method, wire-mesh cages filled with apple tree bark and 20 last-instar CM larvae were used, while different nematode species and concentrations were used as treatments. The cages were kept moist, while temperature and moisture levels were recorded during 24 h in the field, after which they were retrieved, and the CM larvae removed and washed. After four days, infection was confirmed by dissection. Five *S. yirgalemense* concentrations and three nematode species (*Steinernema yirgalemense*, *S. feltiae* and *Heterorhabditis bacteriophora)* were investigated. *Steinernema yirgalemense* caused the highest level of mortality of CM larvae, with no significant difference being found between *S. yirgalemense* concentrations investigated.

Contributed paper. Thursday, 9:15. **212-STU**

A new entomopathogenic *Oscheius* (Nematoda: Rhabditidae) from Italian cave

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Specimens of nematode belonging to *Oscheius* genus was isolated through the *Galleria* bait method from soil collected

in a karst cave of Tuscany (Central Italy). Molecular and morphological analyses were performed. Total DNA was extracted from individual nematodes and the mitochondrial COI, the ITS containing region and the 18S rRNA gene were amplified and sequenced. BLAST search at NCBI discriminate this new taxon, similar to other *Oscheius*. This species belongs to Dolichura group. Cuticle is finely annulated, stoma is short and cheilorhabdion is simple, not well cuticularized. Female body is almost straight upon fixation, the reproductive system is didelphic and tail is short, conoid with pointed tip. Males are rare and similar to female in general morphology except for smaller size. Male body is straight when heat-killed, testis is single, ventral reflexed. They show peloderan bursa, tail short rounded and spicules slender and small. Infective Juveniles are slender with elongate tail and have stoma morphology similar to adult. The nematodes were cultured in Petri dishes on several substrates: Nutrient Agar, *Escherichia coli*, *Botritis cinerea*, meat baby food, without satisfactory results. Only Petri dishes method with *G. mellonella* larvae produced IJs, suggesting the entomopathogenicity of this new taxon.

Contributed paper. Thursday, 9:30. **213**

Genetic improvement of the entomopathogenic nematode *Heterorhabditis bacteriophora*

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Abstract-The entomopathogenic nematode *Heterorhabditis bacteriophora* has been genetically improved in beneficial traits, like heat and desiccation tolerance, by cross breeding and genetic selection. For instance, a final overall increase in mean heat tolerance of 5.5°C was achieved with *Heterorhabditis bacteriophora* by cross breeding the most tolerant five strains and then selecting for heat resistance. Success of breeding programmes largely depends on the heritability of the investigated traits. Advances in enhancement of desiccation and heat tolerance often have been lost again during mass production. For heterorhabditid nematodes methods have now been developed to stabilize the traits by selection of tolerant inbred lines. This technique provides a pathway to genetic improvement of commercial strains which will maintain the improved characters also during in vitro mass production. The methodology to produce stable inbred lines for steinernematids needs further investigation, as these nematodes are amphimictic and production of inbred lines is much more laborious. The reproduction potential in liquid culture was also successfully increased. Future targets for genetic improvement are prolongation of shelf life and field persistence and enhancement of virulence.

Contributed paper. Thursday, 9:45. **214-STU**

Perspectives of new nematode formulation technology for biological control to pest insects in Georgia

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In the result of route investigations the soil samples for searching of entomopathogenic nematodes (EPNs) have been collected in several agrocenosis of different regions of Georgia. Samplings of testing material were done by using of recent methods in insect nematology (Stock & Goodrich-Blair, 2012). According to preliminary data some active strains of *Steinernema* sp. have been obtained. EPNs extract efficiency was established on laboratory culture of *Galleria mellonella*. Further research directions for the identification of local strains (under the Project CRDF/DTRA/GRDF #GMG-01/13) have been conducted at the University of Arizona, laboratory of Entomology by two different ways: morphological and molecular diagnostic methods. It was established that four local EPNs isolates belong to the genus *Steinernema*. Furthermore partial sequencing of the ITS rDNA gene revealed they are closely related to the species *Steinernema feltiae*. This conventionally called - *"*Georgian strain", considered as a raw material will be base for local production of bioformulation -*"Geo-nema"*. Provided technological product - environmentally safe nematode insecticide will be used for biological control to the pest insects of agricultural crops and ornamental plants. The researches will be continued under the projects CRDF/STEP and SRNSF/STCU financial support. The usage of nematode insecticide will take an important place in IPM (integrated pest management) system for agricultural crop protection in Georgia.

CONTRIBUTED PAPERS Thursday, 8:00-10:00

Viruses 6

Contributed paper. Thursday, 8:00. **215**

Interactions between salivary gland hypertrophy virus and tsetse microbiota

Güler Demirbas Uzel [1], Vangelis Doudoumis[2], Antonios Augustinos[1], Gisele Ouedroogo[1], Andrew Parker[1], Drion Boucias[3], Kostas Bourtzis[1], Adly Abd-Alla[1],
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Many species of tsetse flies are infected by a hytrosavirus that causes Salivary Gland Hypertrophy (SGH) syndrome. Flies with SGH have a reduced fecundity and fertility. Due to the deleterious impact of the salivary gland hypertrophy virus (SGHV) on *Glossina pallidipes* colonies, several approaches have been investigated to develop a virus management strategy including the exploitation of endogenous microbiota. Tsetse flies harbor three symbiotic bacteria *(Wigglesworthia glossinidia, Sodalis glossinidius* and *Wolbachia*) in addition to trypanosome, the causative agent of sleeping sickness disease in human and nagana in livestock. The interaction of the tsetse microbiota (gut bacteria and symbionts) with the SGHV and / or trypanosome is largely unexplored. In the present study, we show that ampicillin treatment of *G. pallidipes* impedes the transgeneration transmission of the SGHV suggesting the involvement of tsetse microbiota in the virus transmission. Quantitative-PCR analysis of the levels of SGHV and *Wolbachia* in wild tsetse flies (mainly *G. morsitans morsitans* and *G. austeni*) clearly indicated a negative interaction between SGHV and *Wolbachia*: flies heavily infected with *Wolbachia* presented significantly low viral titers. In addition, injection of GpSGHV into different *Wolbachia*-infected *Glossina* species did not result to the transgeneration transmission of SGHV as normally occurs in *G. pallidipes* colony, which is free of *Wolbachia*. Taken together, these data

suggest that *Wolbachia* may interfere with the establishment and transmission of this important DNA virus (SGHV), which represents a major hurdle for the application of SIT strategies for the control of tsetse flies and trypanosomosis in sub-Saharan Africa.

Contributed paper. Thursday, 8:15. **216-STU**

Mechanisms of tree-top disease induced by the specialist baculovirus SeMNPV

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Many parasites alter host behavior to enhance their transmission or survival. An intriguing example is the altered behavior of insect larvae infected by a baculovirus, e.g. their movement to elevated positions. This phenomenon (tree top disease or Wipfelkrankheit) is already known for over a century. However, the underlying mechanisms leading to this behavioral adaptation are still largely enigmatic. Here we studied tree-top disease induced by the baculovirus *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) in *S. exigua* larvae. We show that infected *S. exigua* caterpillars all climb to elevated positions prior to death. Furthermore, we investigate the role of the ecdysteroid UDP-glucosyl transferase (*egt*) gene from SeMNPV in tree-top disease. This gene is known to be important in tree-top disease in another baculovirus-host system, although the mechanism by which it exerts this effect is unknown. We hypothesize that the SeMNPV *egt* gene may directly trigger tree-top disease or induce this phenomenon indirectly by prolonging the larval time to death.

Contributed paper. Thursday, 8:30. **217**

Temporal proteomics to study virus infection and function in the host cell

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Invertebrate iridescent virus 6 (IIV-6) is a nucleocytoplasmic virus with a 212 kb-long linear double-stranded DNA genome that encodes 215 putative open reading frames. The IIV-6 virion proteome consists of at least 54 virally-encoded proteins. One of our previous findings showed that most of these proteins are encoded by genes from the early transcriptional class. This indicates that these structural proteins may not only function in the formation of the virion, but also in the initial stage of viral infection. In the current study, we followed the protein expression profile of IIV-6 over time in Drosophila S2 cells by label-free quantitation using nanoLC-FTMS. A total of 95 viral encoded proteins were detected in infected cells, of which 37 are virion proteins. The expressed IIV-6 virion proteins could be categorized into three main clusters based on their expression profiles. These clusters were: 1) proteins with stably low or 2) exponentially increased expression levels during infection, and 3) proteins that were initially highly abundant, and then showed slightly reduced levels after 48 hours (h) post infection (p.i.). The study supported that temporal expression patterns did not share direct correlation with protein expression classes phenomena, suggesting that both proteomic and transcriptomic approaches will be required to obtain a detailed understanding of the viral expressomics (infectome). Here, we provide novel information on the kinetics of virion and infected cell-specific protein levels that assists in understanding gene regulation in this lesser known DNA virus model.

Contributed paper. Thursday, 8:45. **218**

Characterization of an atypical fast-killing ascovirus: *Spodoptera frigiperda* ascovirus 1d (SfAV-1d)

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Ascoviruses (AVs) are large double-stranded DNA viruses that attack lepidopterans, mainly noctuid larvae. One of the unusual features of AVs is their mode of transmission via parasitoid wasps. AVs are poorly infectious *per os* compared to other insect viruses such as baculoviruses and cypoviruses. Additionally, AV infection results in production of a characteristic milky-white hemolymph due to accumulation of virion-containing vesicles produced by a modified apoptotic response in infected cells. Virtually all ascoviruses cause a chronic disease wherein larvae survive for as long as 28 days after infection, which enables an extended period of transmission among larvae by wasps. Here, we report characterization of *Spodoptera frugiperda* ascovirus 1d (SfAV-1d) isolated from a *S. frugiperda* larva. SfAV-1d killed *S. litura* 4th instar larvae within 3 days when compared to another AV (SfAV-N), which took as long as 23 days to kill larvae. Larvae infected with SfAV-1d contained the characteristic white hemolymph. Electron microscopy revealed that both SfAV-1d and SfAV-N infected the fat body but not the tracheal matrix or other tissues. Interestingly, despite the difference in the rate at which SfAV-1d and SfAV-N killed larvae, there was no apparent difference in the kinetics of viral DNA replication. The primary difference between these two isolates was that SfAV-1d formed and accumulated virion-containing vesicles in the hemolymph much more rapidly than SfAV-N. Our future studies will focus on characterizing the genetic differences between these viruses to identify determinants that influence their pathobiology, particularly as it relates to rate of kill.

Contributed paper. Thursday, 9:00. **219-STU**

Two nucleopolyhedroviruses isolated from the genus *Adoxophyes* inhibit juvenile hormone (JH) esterase activity but not JH epoxide hydrolase activity

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Insect metamorphosis is predominantly regulated by two hormones, juvenile hormone (JH) and ecdysone. During the final instar, a dramatic decrease in JH titer is required for the induction of pupation. JH is metabolized by two enzymes, JH

esterase (JHE) and JH epoxide hydrolase (JHEH). *Adoxophyes honmai* (Lepidoptera: Tortricidae) is susceptible to two nucleopolyhedroviruses (NPVs), *A. honmai NPV* (AdhoNPV) and *A. orana NPV* (AdorNPV), which are genetically closely related but differ in killing speed. AdhoNPV kills the host only in the final instar, whereas AdorNPV kills more quickly (5 to 8 days). When 4th instars of *A. honmai* are orally inoculated at >LC_{95} (1.0 x 10^8 OBs/ml), AdhoNPV and AdorNPV prevent pupation and kill the host in 10 and 8 days, respectively. In contrast, mock-inoculated larvae pupate in 7 days. Baculoviruses are known to prevent pupation through endocrynological regulation. Here, we monitored both JHE and JHEH activities in AdhoNPV-, AdorNPV-, and mock-infected larva of *A.honmai*. Mock-infected larvae showed increased JHE activity in the hemolymph and fat body during the final instar, with the highest activity found on the 3rd day of the 5th instar. Both AdhoNPV- and AdorNPV-infected larvae did not show JHE activation. On the other hand, JHEH activity in fat body was constant and no differences were found between treatments. Our data suggest that AdhoNPV and AdorNPV prevent pupation by specifically down-regulating JHE but have no effect on JHEH activity. Our data also suggest that JH titers remain relatively high during the final instar of baculovirus infection.

Contributed paper. Thursday, 9:15. **220**

Mechanism underlying virus-induced hyperactive behavior: Substrate identification of the baculovirus protein tyrosine phosphatase

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Many parasites alter the behavior of their host to maximize their transmission and survival. However, the underlying mechanisms are largely unknown. Baculoviruses manipulate the behavior of their caterpillar hosts by inducing hyperactivity and climbing behavior. Previous work demonstrated that a protein tyrosine phosphatase (PTP) encoded by the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) was involved in the induction of hyperactive behavior in *Spodoptera exigua* larvae. This finding prompted us to investigate which viral and/or host proteins interact with the baculovirus PTP enzyme and might be involved in altered host behavior. Using affinity-tag purification of a substrate-trapping mutant of AcMNPV PTP incubated with extracts of infected cells followed by proteomic analysis of the trapped protein, we identified six viral and six host proteins that co-purified with PTP. Several of these proteins are known to be important in cellular signaling and in behavior in other insects/organisms, and are therefore potentially involved in PTP-mediated hyperactivity of infected larvae. For one of these identified host proteins, the 14-3-3 ζ protein, RNA expression levels were significantly higher for AcMNPV wild type-infected larvae as compared to AcMNPV Δ*ptp*-infected larvae, indicating that *14-3-3 ζ* expression levels are dependent on the presence of the baculovirus *ptp* gene. The 14-3-3 ζ protein is known to be important for the synthesis of serotonin and dopamine, which are neurotransmitters that play important roles in many behavioral pathways. It is hypothesized that baculovirus *ptp* targets 14-3-3 ζ at both the RNA and protein level, which consequently leads to baculovirus-induced hyperactivity.

Contributed paper. Thursday, 9:30. **221-STU**

The genome of a baculovirus isolated from *Lonomia obliqua* (Lepidoptera:Saturniidae) reveals a new transcription terminator factor possible acquired from the host

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Lonomia obliqua (Lepidoptera: Saturniidae) is a poisonous larvae of medical importance due to the severity of accidents occurred in Brazil caused by the contact of these larvae with the human skin. The possibility of controlling these populations is being evaluated by using pathogens such as a nucleopolyhedrovirus isolated from *L. obliqua*. In this work, we have sequenced the genome of the baculovirus *Loob*MNPV and analyzed its genomic composition and evolutionary history. The genome is 120.022 bp long, comprising 135 putative ORFs. Furthermore, in an evolutionary context, based on analysis that include the core gene from 93 sequenced baculovirus , *Loob*MNPV fell into a basal position related to the *Alphabaculovirus* group I (lepidopteran-infective NPV). Interestingly, one ORF showed significant identity (*e-value* equals to $3e10^{-11}$) to a eukaryotic transcription terminator factor (TTF2) from the lepidoptera *Danaus plexippus* (GenBank: EHJ68439.1). On the other hand, when restricting this search only to baculoviruses, this ORF also demonstrated identity (*e-value* of $1e10^{-6}$) to the Global Transactivator (GTA) gene from *Antheraea pernyi* nucleopolyhedrovirus (Genbank:YP_611073.1). Phylogenetic analysis were performed with the TTF2 gene from various organisms, as well as with the GTA from baculovirus. These results indicated two hypothesis: (i) this gene may have been independently acquired from the host through horizontal transfer, acting as an inhibitor of the host´s transcriptional machinery in order to benefit viral translation; (ii) or it is a divergent variation of the GTA gene that has undergone positive selection.

Contributed paper. Thursday, 9:45. **222**

The essential baculovirus protein VP1054 is a hijacked cellular PURα,
a nucleic-acid-binding protein specific for GGN repeats

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The baculovirus VP1054 protein is a structural component of both budded virus (BV) and occlusion-derived virus (ODV), but its exact role in virion morphogenesis is poorly defined. We reveal sequence and functional similarity between the baculovirus protein VP1054 and the cellular *pu*rine-*r*ich element binding protein PUR-alpha (PURα). The data strongly suggest that gene transfer has occurred from a host to an ancestral baculovirus. Deletion of the AcMNPV *vp1054* gene completely prevented viral cell-to-cell spread. Electron microscopy data showed that assembly of progeny nucleocapsids was dramatically reduced in the absence of VP1054. More precisely, VP1054 is required for proper viral DNA encapsidation, as deduced from the formation of numerous electron-lucent capsid-like tubules. Complementary searching identified the presence of genetic elements composed of repeated GGN trinucleotide motifs in baculovirus

genomes, the target sequence for PURα proteins. Interestingly, these GGN-rich sequences are disproportionally distributed in baculoviral genomes and mostly occurred in proximity to the polyhedrin gene. At the same time they encode crucial proline-rich domains in *p78/83*, an essential gene adjacent to the *polyhedrin* gene in the AcMNPV genome. We further demonstrate that the VP1054 protein specifically recognizes GGN-repeats and are currently analyzing the significance of these GGN motifs for DNA packaging. Together, whilst some viruses like human immunodeficiency virus 1 (HIV-1) and human JC virus (JCV) utilize host PURα protein, baculoviruses encode the PURα-like protein VP1054, which is crucial for viral progeny production.

SYMPOSIUM (Special) Thursday, 8:00-10:00

DFG Priority Program Host Parasite Coevolution

Symposium. Thursday, 8:00 **223**

Escaping parasite manipulation: Apoptosis and host-parasite co-evolution in *Apis mellifera*

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Programmed cell death (apoptosis) does not only play an important role in the development of multicellular organisms, but also in the protection against pathogens. Nevertheless, numerous intracellular pathogens have evolved diverse strategies to interfere with and overcome the apoptotic machinery of their hosts. Yet, little is known about the actual mechanisms and how hosts might counter act. We here study the interaction of the intestinal microsporidian parasite *Nosema ceranae* in a susceptible and tolerant honeybee host under laboratory controlled conditions, to understand the importance of apoptosis in this case of host-parasite co-evolution. We visualize apoptotic processes in the gut epithelium using TUNEL assays; relate this to the expression levels of key genes in the apoptotic cascade over the course of the infection, and consequences for metabolic energetics affecting honeybee performance.

Symposium. Thursday, 8:15 **224**

Overcoming external immunity: An increase in virulence as a result of host-parasite coevolution in *Beauveria bassiana*

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An increase in virulence is a trait often observed as a result of host-parasite coevolution. Specific immune responses overcome in order to achieve increased virulence can, however, be difficult to elucidate. We carried out a coevolution experiment with the red flour beetle, *Tribolium castaneum*, and the general entomopathogenic fungus, *Beauveria bassiana*. After just seven host generations of evolution we saw a substantial increase in virulence in all evolved isolates of *B. bassiana*. Furthermore, we were able to show that this increase in virulence was a result of the *B. bassiana* isolates evolving resistance to the external immune defences of the *T. castaneum* beetles, who are able to secrete antimicrobial compounds into their environment. This is a rare example of a virulence increase seen as a result of a coevolution experiment where the exact barrier of host immune defence that the parasite has gained resistance to in order to achieve the increase in virulence has been described.

Symposium. Thursday, 8:30 **225**

Rapid adaptation of *Bacillus thuringiensis* to its nematode host *Caneorhabditis elegans*

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Antagonistic interactions between host and pathogen can produce very high selection intensities. They are often one of the main driving forces during evolution, especially if the interactions persist across time. We specifically assessed the evolutionary impact of these interactions for the pathogen, using evolution experiments with the Gram positive biocontrol agent *Bacillus thuringiensis* and one of its animal hosts *Caenorhabditis elegans*. Our results demonstrate that differences in the experienced selection conditions during the evolution experiment favour distinct characteristics across the pathogen life cycle: (i) pathogen adaptation to a co-evolving host associates with high virulence; (ii) pathogen adaptation to a non-changing host increases infection load; whereas (iii) adaptation without host favours environmental persistence. Concomitant genomic changes in the pathogen were observed at two levels: (i) the different evolution conditions caused clonal selection of distinct, broad-scale genotypes, while (ii) one of these with high virulence showed additional nucleotide changes, including copy number variations of nematocidal toxin genes. Based on one of the most comprehensive data sets collected for an experimentally evolved pathogen, we conclude that sustained coevolution is distinct from other types of selective constraints in shaping pathogen genome and life-history characteristics. Surprisingly, our findings also suggest that sustained virulence, as desired for pest control, may be contingent on the unwanted co-adaptation of the target host.

Symposium. Thursday, 8:45 **226**

Intra-host parasite interactions between co-infecting *Bacillus thuringiensis* strains

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Hosts and parasites are expected to influence each others evolution due to antagonistic interactions, potentially leading to host-parasite coevolution. However, many studies focus on the interactions between hosts and parasites, ignoring that within one host different parasite genotypes may interact and may thus feed-back on the coevolution between host and parasite. The interactions between parasite genotypes may range from competition between genotypes for limited host resources to cooperation for more efficient host exploitation. Using *Caenorhabditis elegans* as host and the bacterial microparasite *Bacillus thuringiensis* we found indications for diverse interaction strategies between the bacteria, ranging from public good to spiteful bacteriocin production. However, it remains unclear how stable these strategies are over the course of time, i.e. when hosts have to be repeatedly infected and when hosts may also adapt to these parasite strategies. For this reason, we performed a laboratory-based selection experiment in which either single *B. thuringiensis* genotypes or a mixture of strains coevolved with hosts. After 10 host generations, we found differences between the evolution treatments. Most interestingly, mixed infections strongly lost virulence. Whether this is caused by a trade-off between host-exploitation and bacterial competition or by division of labour between bacterial clones remains to be shown. Importantly, these results have strong implications for epidemiology, since the evolution of bacteria and its consequences for the host depend on the multitude of infection.

Symposium. Thursday, 9:00 **227**

Experimental evolution *in silico*: host-parasite coevolution versus parasite adaptation

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Bacillus thuringiensis is a widely distributed natural pathogen of invertebrates and plays an important role in (agricultural) pest management. The bacteria kill the host by CRY toxins and other virulence factors. Recent experimental studies on the evolution of virulence revealed that one-sided adaptation of *B.t.* with non-evolving hosts (*Caenorhabditis elegans*, *Tribolium castaneum*) selects for intermediate or no virulence, sometimes coupled with parasite extinction. In contrast, host-parasite co-evolution selects for high virulence and for hosts with strong resistance against *B.t.* However, a sound theoretical explanation is missing. Here, we propose a new mathematical model that mimics the experimental set-up. We consider two bacterial strains, a virulent “toxin producer” and an avirulent “non-toxin producer”. Bacterial evolution is modeled as an iterated process of intra-host dynamics and bacterial transmission between hosts. The intra-host dynamics are described as a two-phase process, where the first phase covers the period from beginning of infection until host death and the second phase the period from host death until depletion of host resources. Increase in host resistance is simulated by extending the first phase. Our model analysis revealed, in general, the same basic trends as the above-mentioned experimental studies. Especially, we could show that resistant hosts select for highly virulent bacterial strains. Moreover, we found (1) that the evolved level of virulence is independent of the initial level of virulence, and (2) that the bacterial dosage significantly affects the evolution of virulence with low dosage selecting for highly virulent strains. These predictions can be tested in future experiments.

Symposium. Thursday, 9:15 **228**

Immune priming with *Bacillus thuringiensis* in *Tribolium castaneum*

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There is accumulating evidence for a memory-like phenomenon in the immune defence of invertebrates. Such 'immune priming' can be rather specific, and might be transmitted from parents to offspring. Invertebrates do not possess the machinery of the vertebrate adaptive immune system, and the mechanistic underpinnings of immune priming are still largely unknown. In the red flour beetle *Tribolium castaneum*, immune priming for resistance against the entomopathogen *Bacillus thuringiensis* has been demonstrated, both within and across generations. Immune priming arose after septic 'pricking' as well as oral pathogen exposure. Moreover, not only mothers, but also fathers were able to transmit such resistance to their offspring. In this talk I will present our recent approaches to deepen our understanding of the evolutionary relevance and mechanistic underpinnings of immune priming in this host-pathogen system.

Symposium. Thursday, 9:30 **229**

Rapid reciprocal adaptation between the red flour beetle and *Bacillus thuringiensis* bacteria during experimental coevolution

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The antagonistic interaction between hosts and parasites is a powerful evolutionary force that may drive rapid evolutionary adaptation. It can lead to coevolution by reciprocal adaptation and counter-adaptation of hosts and parasites. However, in natural populations, it is very difficult to exclude other selective forces that may influence the interaction and to identify true coevolution. We thus performed experimental coevolution in the laboratory between the red flour beetle and *Bacillus thuringiensis* bacteria. We made use of an experimental design that included control treatments in which either of the antagonists was allowed to adapt to a non-evolving host or parasite, respectively, and we also controlled for a possible adaptation to laboratory conditions. We here report on evolved differences in the phenotypes of host and parasite,

and in particular an observed increase in parasite virulence and host resistance. Moreover, we found a potential for parasite local adaptation under coevolution.

Symposium. Thursday, 9:45 **230**

Means of fast virulence adaption: the plasmid and prophage equipment of selected *Bacillus thuringiensis* strains

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Strains of *Bacillus thuringiensis* (Bt) are used since decades as pest control in crop protection. A descriptive feature of the species is the existence of paracrystal bodies, which consist of δ-endotoxins, acting against specific classes of invertebrates. Over the years a solid amount of research has been achieved on the activity of δ-endotoxins on invertebrates as well as on the diversity of cry-toxin genes. In contrast surprisingly little is known on the genomic loci which encode this diversity of δ-endotoxins. Furthermore the knowledge on other invertebrate virulence factors encoded by Bt as well as on host adaptation factors is rather fragmentary. The observation of phenotypes that differ between strains indicates that they are encoded within the pan-genome of *Bacillus thuringiensis*. Since a pan-genome consists of the genes that are not shared by all members of species many of them are encoded on strain specific extra chromosomal elements. Here we present a comparative analysis of more than 40 extra chromosomal replicons such as plasmids and prophages of three nematocidal and two insecticidal Bt strains.

SYMPOSIUM 8 (Cross-Divisional) Thursday, 14:00-16:00

Host – Pathogen Ecology at the Molecular Level: Gene Regulation and Environment Sensing

Symposium. Thursday, 14:00. **231**

The *Bacillus thuringiensis* way of life: communicate to kill and survive in the insect host

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At the end of exponential growth, bacteria of the *Bacillus cereus* group (*ie. B. thuringiensis* and *B. cereus*) produce virulence factors allowing the bacteria to invade their host. In the insect gut, genes controlled by the PlcR quorum sensor allow the bacteria to damage the intestinal barrier and to gain access to the haemocoel. After the death of the insect, PlcR activates transcription of a gene encoding a second quorum sensor, NprR. NprR induces production of degradative enzymes and of a biosurfactant allowing the bacteria to survive in the insect cadaver and eventually to sporulate. The development of the sporulation process is controlled by the master regulator Spo0A, whose activity is regulated by Rap proteins. PlcR, NprR and Rap are quorum sensing regulators belonging to the RNPP family. Their activity depends on the signalling peptides PapR, NprX and Phr, respectively. Altogether our results indicate that these three cell-cell communication systems, acting sequentially, coordinate virulence and adaptive properties with the general physiology of the bacterial cells. The PlcR-PapR complex induces the production of virulence factors allowing the bacteria to kill the insect. NprR-NprX activates transcription of genes allowing the bacteria to switch from a virulence state to that of survival in the host cadaver. Ultimately, the inhibition of the Rap proteins by the Phr signalling peptides triggers sporulation, thus allowing the bacteria to disseminate and to persist in the environment.

Symposium. Thursday, 14:30. **232**

The interplay of Paenibacillus larvae with honey larvae during infection

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Honey bees are attacked by numerous pathogens, some of them just causing covert infections others causing overt disease symptoms and even death of individuals and entire colonies. Among the latter group is the bacterium *Paenibacillus larvae*, the etiological agent of the epizootic American Foulbrood of honey bees (AFB). As the name suggests, AFB is a bacterial disease affecting only the larval stages of honey bees. *P. larvae* is an obligate killer because death of larvae and conversion of larval biomass into bacterial biomass are prerequisites for disease transmission within and between colonies. Hence, *P. larvae* must have evolved effective means to attack larvae, to circumvent the larval immune response and to finally kill and decompose larvae. We recently identified and characterized some of these virulence factors of *P. larvae*. We will present a model for molecular pathogenesis of *P. larvae* infections built upon these novel findings in order to further the understanding of the molecular basis of pathogen-host-interactions in American Foulbrood disease.

Symposium. Thursday, 15:00. **233**

Antimicrobial defense and persistent infection in insects revisited

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Antimicrobial peptides are mainly produced and used by multicellular organisms such as insects to interact with pathogenic and mutualistic micro-organisms. Antibiotics are mostly produced by single cell eukaryotes and bacteria. Here we provide a possible explanation for this dichotomy. Our hypothesis is based on the observation that antibiotics elevate bacterial mutation rates and we show that AMPs do not elevate bacterial mutation rates. Nevertheless we also found that bacterial resistance evolves readily against single AMPs in vitro, but the situation is already more complicated by the simultaneous action of two AMPs. I will contextualize these findings in the light of the immune responses of the beetle Tenebrio molitor and will use these findings to discuss some of the multiple roles AMPs have in host-microbe interactions: policing and killing.

Symposium. Thursday, 15:30. **234**

Vibrio and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes

Audrey Vanhove [1], Annick Jacq [2], Frédérique Le Roux [3], Tristan Rubio [1], Alexandra Calteau [4], Evelyne Bachère [1], Julie Nicod [1], Agnès Vergnes [1], Astrid Lemire [3], Guillaume Charrière [1] and Delphine Destoumieux-Garzón [1]
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Vibrio tasmaniensis LGP32 is a *V. splendidus*-related strain pathogenic for *Crassostrea gigas* oysters. We recently showed that LGP32 invades the oyster immune cells, the hemocytes, through phagocytosis. Oyster hemocytes are professional phagocytes harboring microbicidal activities including a potent oxidative response. Interestingly, the phagocytosed LGP32 survives inside the oyster hemocytes, evading the host defense by preventing acidic vacuole formation and limiting reactive oxygen species production. When hemocytes were invaded by numerous LGP32, we observed cytotoxic effects such as membrane disruptions and cytoplasmic disorders. Cytotoxicity was shown to entirely depend on LGP32 entry into hemocytes, as cytochalasin D was sufficient to inhibit hemocyte death. By developing a transcriptomic approach based on RNA sequencing, we identified a series of *Vibrio* antioxidant genes whose expression is strongly induced within oyster hemocytes. We also observed an overexpression of genes involved in cation efflux. Overexpression of these molecular functions in the intraphagosomal stage was confirmed by RT-PCR.To determine how far those LGP32 genes are involved in resistance to intracellular killing and subsequent virulence, we constructed isogenic deletion mutants for two overexpressed antioxydants and two overexpressed cation transporters. Those mutants were phenotyped for intracellular multiplication, cytotoxicity and virulence in oyster experimental infections. Our data show that resistance to reactive oxygen species and efflux of cations are two important functions required for LGP32 intracellular survival, cytotoxic effects and virulence.

CONTRIBUTED PAPERS Thursday, 14:00-16:00

MICROBIAL CONTROL 4

Contributed paper. Thursday, 14:00. **235**

Establishing the fungal entomopathogen _Beauveria bassiana_ (Ascomycota: Hypocreales) as an endophyte in cucurbits for managing Zucchini Yellow Mosaic Virus (ZYMV)

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The fungal entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is known to survive as an endophyte in a wide range of plants and offer protection against an increasing number of insect pests. Although recent discoveries suggest that the fungus can also protect plants against plant pathogens, no studies are currently available on the efficacy of endophytic *B. bassiana* against plant viruses. We conducted experiments to determine whether endophytic *B. bassiana* could provide protection against Zucchini Yellow Mosaic Virus (ZYMV), one of the most economically important diseases of cucurbits worldwide. Four selected *B. bassiana* strains were able to successfully colonize squash plants following the foliar inoculation of plants with the conidial suspension of each respective strain. Disease incidence and severity, sampled weekly following the challenge inoculation of plants with ZYMV, were significantly lower in *B. bassiana*-inoculated plants as compared to control plants; irrespective of the *B. bassiana* strain being inoculated. Our study demonstrates, for the first time, that endophytic *B. bassiana* has the biocontrol potential for managing plant viruses. Further studies should be conducted to determine whether such endophytic *B. bassiana*-mediated protection against ZYMV in squash extends to other cucurbits.

Contributed paper. Thursday, 14:15. **236**

Bean plant _Phaseolus vulgaris_ endophytically colonized by _Beauveria bassiana_ and _Hypocrea lixii_ acquires protection against _Liriomyza huidobrensis_ (Diptera: Agromyzidae) in the field

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Field trials were carried out for two cropping seasons in two sites (Sagana and Narumoro, Central province of Kenya) to evaluate the prospects of endophyte isolates of *Beauveria bassiana* and *Hypocrea lixii* for the control of leafminer *Liriomyza huidobrensis* in *Phaseolus vulgaris.* Autodissemination device treated with conidia of *Metarhizium anisopliae* was also added as a treatment. The effects of endophytes on leafminer infestation (punctures and mines), number of pupae and parasitoids, and yield were evaluated. Both isolates successfully colonized different parts of *P. vulgaris* plants; however, colonization was greater with *H. lixii* than *B. bassiana* in both sites. Leafminer infestation was not significantly different during the first season while it was higher in the controls than in endophtyte treatments at both sites during the second season. The number of pupae varied between 150-250 and 320-400 in endophyte and control treatments, respectively, during the first season; and from 100-200 and 350-500, respectively, in endophyte and control treatments during the second season. The number of parasitoids that emerged from pupae did not differ significantly among the treatments. Higher yield was obtained in endophyte than in control treatments. With exception to yield during season two, the inclusion of autodissemination device treatment did not have significant effect on all the parameters evaluated. There were no significant differences between the fungal isolates. Results of the present study suggest that both endophyte fungal isolates hold potential and could be considered for the control of leafminer. There is the need however to confirm these results on large-scale trials.

Contributed paper. Thursday, 14:30. **237**

Colonized plants with entomopathogenic fungi produce mortality in *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae

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This study aimed to evaluate the ability of entomopathogenic fungi to colonize endophitically plants for *Spodoptera littoralis* (Boisduval) (Lepidoptera, Noctuidae) larvae control, which is a polyphagous insect of economic importance with a wide range of host plants. The five isolates of *Beauveria* (3) and *Metarhizium* (2) (Ascomycota, Hypocreales) were able to colonize endophytically tomato (*Lycopersicon esculentum* Mill), melon (*Cucumis melo* L.*,* hybrid F1- Galia) and alfalfa (*Medicago sativa*) plants. The tissues colonization of the evaluated plants was determined by the fungus re-isolation of leaves, stem and roots. Two fungal strains, EAMb 09/01-Su and Bb04, showed an increasingly colonization presenting from 4.0 to 24.3 % of colonization of the root tissues by 24 to 96h, and 43.3 to 98.0 % of stem and leaves by 24 to 72h. The potential of this fungus as a mycoinsecticide for the control of *S. littoralis* was also evaluated in present study. In the first step, the larval mortality was determined after topical application of conidial suspension of higher virulent isolates, which showed mortality percentage of 41.6% for EAMb 09/01-Su and 76.6% for EABb 01/33-Su. The ingestion by larvae of alfalfa leaves colonized endophytically showed a significant larval mortality by 25.0% and 31.6% respectively. No differences in leaf consumption between treatments and controls were found, so the possibility of a repellent or a feeding deterrence effect is not appreciated. In conclusion, this study provides evidence for the ability of fungi to colonize internal tissues of tomato, melon and alfalfa, as well as to control *S. littoralis* larvae.

Contributed paper. Thursday, 14:45. **238**

***Beauveria bassiana* and California strawberries: endophytic, mycorrhizal, and entmopathogenic interactions**

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A greenhouse study in 2010 showed that *Beauveria bassiana* colonized strawberry plants and persisted in various tissues for up to 9 weeks. Another greenhouse study in 2013 evaluated the impact of soil and foliar applications of *B. bassiana* on green peach aphid, *Myzus persicae* feeding on potted strawberry plants. Small plot and field studies in 2013 and 2014 indicate that root or soil treatment with *B. bassiana* promotes the strawberry plant growth and development. Treating the roots of the strawberry transplants with *B. bassiana* before planting significantly improved the plant health compared to untreated control and a microbial plant growth enhancer in a 2013 study. Preliminary data from a field study that is currently under investigation also indicate a positive impact of soil treatment of *B. bassiana* on plant growth. Plant canopy is larger in treated plants compared to the grower standard practices alone. A large strawberry field study in 2013 demonstrated the role of *B. bassiana* in strawberry IPM. Results of various studies will be discussed in exploring the role of entomopathogens in pest management and promoting plant development.

Contributed paper. Thursday, 15:00. **239**

Perceptions, trust, terminology and influence: What do consumers think about biological control?

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Those of us who work in the field of biological control (in the broadest sense, including GM crops and micro-organisms) inherently believe these practices to be environmentally benign and significantly safer than conventional alternatives. We tend to engage in terminology describing the science, even when communicating with the general public, although many are unfamiliar with the field and may have only a rudimentary understanding of the concepts and science behind these technologies. Yet if we are to promote use beyond 'niche' markets and advance broader acceptance, technological developments aside, we need to fully engage the public as key partners driving change. Greater understanding of the consumer mind-set allows us to communicate concepts more effectively, and to potentially use biocontrol to positively influence purchase decisions. Here, data from several consumer studies will provide insights into general perceptions of biological control and how these are influenced by trust in science and technology; considerations when phrasing pest management practices to communicate information to consumers; and effects of pest management practices on the likelihood of consumers' purchasing floral or edible crops grown using different pest management practices. Within the parameters of the study, price and pest management practice were consistently the most important factors influencing consumer purchase intention. Findings highlight the importance of using every-day terms when engaging the general public, but also clearly show that there are opportunities to positively influence peoples' choices for products grown using 'natural' methods – as long as we can talk with them in a language they understand.

Contributed paper. Thursday, 15:15. **240**

A phylogenetic survey of protistan parasites

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The advent of molecular biology techniques has shown that parasitism has evolved many times in protists and that all of the eukaryotic supergroups contain several major radiations of parasites. It is hypothesised that much of the vast diversity revealed by environmental sequencing studies also derived from so far uncharacterised parasites; evidence in support of this hypothesis is growing. Some parasitic lineages are relatively well known and the subject of research foci, both at the level of individual taxa and of emerging groups that are being studied for their evolutionary interest. However, other lineages, although known to harbour a significant diversity of parasites, are rarely studied or factored into ecological and parasitological studies of potential hosts. This talk will review the diversity of parasites across the eukaryotic tree of life as a whole, and point to groups that are perhaps worthy of

increased attention and vigilence, as well as underlining the range of parasites expected in many systems.

Contributed paper. Thursday, 15:15. **241**

Bacillus thuringiensis toxins vs baculovirus: differential induction of immune system related genes in _Spodoptera exigua_

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Spodoptera exigua Hübner is a polyphagous pest native from Asia that has been spread worldwide. It is a major threat not only for field or flower crops, but also for greenhouse vegetable cultivations. To reduce losses due to *S. exigua* damage, growers often opt for biological control, such as using insecticidal products based on *Bacillus thuringiensis* Berliner (Bt) or baculovirus. Both pathogens act by ingestion and lead to insect death within few days. To counteract the infection, *S. exigua* relies on its immune system response, and production of antimicrobial peptides (AMPs) and proteins is an important part of the innate immune defense cascade triggered by pathogens. In this study, *S. exigua* transcriptome was mined for the presence of unigenes encoding for AMPs and lysozymes, resulting in the identification of a wide and diverse spectrum of these types of defense molecules. Then we compared their transcript abundance in larval midguts after ingestion of different Bt toxins (such as Cry1C and Vip3Aa) or *S. exigua nucleopolyhedrovirus* (SeMNPV) occlusion bodies. Results showed that both Bt proteins triggered a similar pattern of response, which included the specific overexpression of around 80% transcripts tested. In contrast, after SeMNPV ingestion, expression of AMPs decreased or did not change. The possible meaning of *S. exigua* physiological response to different pathogens employed in biological control is discussed.

CONTRIBUTED PAPERS Thursday, 14:00-16:00

VIRUSES 7

Contributed paper. Thursday, 14:00. **242**

Lysine residues in N-terminal tail of a viral histone H4 are crucial in controlling host gene expression

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An endoparasitoid wasp, *Cotesia plutellae*, parasitizes young larvae of the diamondback moth, *Plutella xylostella*. Parasitized larvae undergo significant immunosuppression and developmental alteration. Various parasitic factors have been identified from a polydnavirus, *C. plutellae* bracovirus (CpBV), and teratocytes. A viral histone H4 is identified from CpBV episomal genome. It encodes 141 amino acid residues and shares high sequence homology (82.5%) with host histone H4. Its extended N-terminal region (38 residues) contains 9 Lys residues. Pull-down assay showed that CpBV-H4 interacted chromatin remodeling apparatus, such as SWI/SNF complex. Subtractive suppressive hybridization showed that its expression in nonparasitized host alters the expression of various target genes classified various categories. Indeed, the viral H4 can join to a nucleosome in *in vitro* reconstruction assay. A chromatin immunoprecipitation (ChIP) assay indicates that the viral histone H4s are located at AT-rich regions near to the inducible genes, such as immune, detoxification, and metabolism. The truncated viral histone H4 loses almost inhibitory activity on host immunity. A series of truncated mutants or point mutations at Lys indicate that a specific Lys at 6^{th} from N terminal is crucial to exhibit its epigenetic control of host immunity.

Contributed paper. Thursday, 14:15. **243**

Heat-shock protein 90 is a broadly active regulator for baculovirus infection

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Cellular chaperon Hsp90 plays important roles in diverse biological processes, including signal transduction, protein folding and trafficking, etc. Many viruses, including HCV, HSV and Influenza virus, are dependent on host Hsp90 either for efficient replication or proper intracellular transfer. A recent proteomics study revealed that Hsp90 is incorporated into the budded virions (BVs) of baculovirus, we therefore investigated the role of Hsp90 in the life cycle of baculovirus. By using Hsp90 inhibitor geldanamycin (GA) and RNA interfering, the levels of viral DNA replication, infectious BV production, as well as ODV and polyhedra morphogenesis of baculoviruses were significantly reduced in AcMNPV infected cells. Further studies demonstrated that GA inhibited the expressions of certain viral proteins at transcriptional levels. The nuclear imports of several nucleocapsid- and ODV envelope proteins were also hindered by GA. Interestingly, when the function of Hsp90 was disturbed by GA, virus-triggered nuclear F-actin network essential for assembly of progeny AcMNPV was absent. Taken together, our data suggest that Hsp90 regulates baculovirus replication and morphogenesis from at least three different aspects: 1) promoting the expression of viral proteins; 2) facilitating the intracellular trafficking of viral structural proteins; 3) participating in the nuclear polymerization of host actin which is required for progeny baculovirus production.

Contributed paper. Thursday, 14:30. **244**

Development and immunity-related microRNAs of the lepidopteran model host _Galleria mellonella_

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MicroRNAs (miRNAs) are small non-coding RNAs which have been recognized as key elements in the regulation of protein synthesis at the post-transcriptional level. Our knowledge about their function in regulating complex physiological processes is limited, but rapidly expanding. The larvae of the greater wax moth *Galleria mellonella* have emerged as a powerful and surrogate model hosts for pathogens capable of infecting insects or humans. Complementary to our previously published comprehensive *G. mellonella* transcriptome, here we screened development and immunity-related miRNAs in order to further advance the suitability of this model host. To screen for miRNAs that are differentially expressed in *G. mellonella* either during metamorphosis or upon natural infection with entomopathogenic bacteria or fungi we designed

a microarray spotted with probes of more than two thousand miRNA sequences known from insects. Relative to untreated last instar larvae which were used as a reference, we determined numerous miRNAs to be expressed in prepupae (1037), pupae (981) or pathogen infected last instar larvae (965). Taking advantage of our transcriptomic data base, we were able to identify potential 3' UTRs for determining miRNA-mRNA duplexes by considering both base pair complementarity and minimum free energy (MFE) hybridization. We confirmed the co-expression of selected miRNAs such as miR-71, miR-263a, miR-236b, and their predicted target mRNAs in *G. mellonella* by RTPCR. This is the first study addressing the identification of miRNAs which are predicted to regulate genes that are expressed during metamorphosis or in response to infection of the lepidopteran model host *G. mellonella*.

Contributed paper. Thursday, 14:45. **245**

The *sf122* gene of *Spodoptera frugiperda* nucleopolyhedrovirus modulates key aspects of insect-to-insect transmission and post mortem host liquefaction

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The *sf122* gene present in the longest genotype (SfMNPV-B) of the Nicaraguan isolate of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) was previously identified as undergoing positive selection. A recombinant virus (Sf122null), lacking *sf122,* was generated by homologous recombination from a bacmid comprising SfMNPV-B. Transcriptional analysis revealed that *sf122* is a late gene. Sf122null DNA was two-fold less infective when injected into *S. frugiperda* larvae and occlusion bodies (OBs) of the deletion recombinant were 15-fold less pathogenic (in terms of 50% lethal concentration), speed-of-kill was slower by 20 hours and OB production was reduced 3-fold, compared to the parental virus. The infectious titre of occlusion derived virions (ODVs) of Sf122null was reduced by >100-fold compared to that the parental or *sf122*-repaired viruses. OBs from each virus did not differ significantly in DNA content or gross morphology. Larvae that died from Sf122null infection did not show liquefaction. Similarly, SfMNPV isolates from the United States and Colombia, containing the shorter variant of the protein, only produced partial larvae liquefaction post mortem. Finally, expression of the *chitinase* and *cathepsin* genes was significantly reduced in larvae infected with the Sf122null virus. We conclude that positive selection on the *sf122* gene is most likely related to its marked role in modulating larval liquefaction and virus transmission.

Contributed paper. Thursday, 15:00. **246**

Effect of a Viral Encoded Protein Kinase on Gene Expression in Amsacta moorei Entomopoxvirus Infected Cells

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Insect-born entomopoxviruses (EPVs, Family: *Poxviridae*) are potentially significant biotechnological tools. In comparison to some other insect viruses, the function of relatively few EPV gene has been characterized. In this study, a serine/threonine (Ser/Thr; ORF AMV197) protein kinase gene of the Amsacta moorei entomopoxvirus (AMEV, type species of *Betaentomopoxvirus*) was characterized in terms of regulation of expression relative to some other AMEV genes. A recombinant virus (AmΔPK/*gfp*) was constructed by deleting ORF197 from AMEV genome via homologous recombination. Transcription of wild type virus and recombinant virus genes was compared by whole-genome gene expression microarray. The results showed that the expression levels of 126 genes representing 55.7% of all the viral genes were impacted significantly in the deletion mutant virus. Of these, 88 (69.84 %) transcripts were up-regulated and 38 (30.15 %) were down-regulated. Specifically, transcripts responsible for DNA repair, replication, nucleotide metabolism, and transcription and RNA modification were up-regulated in AmΔPK/*gfp*-infected cells. The results of this study indicate that the product of AMV197 may have significant effects on the assembly and/or infectivity processes of progeny viruses. However, more detail experiments are necessary to identify the exact role of this gene in AMEV replication.

Contributed paper. Thursday, 15:15. **247**

FP25K acts as a negative regulator in the infectivity improvement of AcMNPV Budded viruses

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Baculoviruses can produce two phenotype virions in the replication cycle, the budded virus (BV) and the occlusion-derived virus (ODV). The regulation of forming these two phenotypes virions is an important process in infection, but the mechanism is still unclear. The *fp25k* gene was reported to be responsible for the regulation of BV/ODV formation. The gene mutation results in a decreased number of normal ODV and an increased production of BV. In this study, we unraveled the mechanism of improved infectivity of *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) BVs by *fp25k* deletion. The investigation of BV titer, copy number of BV genome and electro-microscopy observation indicated that the increase of BVs titer of the *fp25k* knock out recombinant is a result of higher infectivity of virions but not the amount of BVs. The identification of protein associated to the virions showed that more BV envelope protein was incorporated into the gene knock out recombinant BVs. However, the infectivity of BVs was confirmed be not increased when GP64 was over expressed in our study. From the transfection and transformation of BV genome DNA into insect cells and *Escherichia*.coli, the results suggested that better integrity genome DNA was packaged in the *fp25k* knock out recombinant BVs. Our study proposed that FP25K is a multifunctional protein in baculovirus life cycle. The virus genome with better integrity might be the major reason of infectivity enhancement and FP25K acts as a negative regulator in this process.

Contributed paper. Thursday, **248**

The leucines in the transmembrane domain of *Autographa californica nucleopolyhedrovirus* Ac76 are important for intranuclear microvesicle formation

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Our previous study has shown that the *Autographa californica nucleopolyhedrovirus* (AcMNPV) *ac76* gene is essential for both budded virion (BV) and occlusion-derived virion (ODV) development. However, the exact role by which *ac76* affects virion morphogenesis remains unknown. In this report, the oligomerization status of Ac76 was investigated and its critical amino acids for intranuclear microvesicle formation were identified to further understand the functional role of Ac76 in virion morphogenesis. Ac76 contains an α-helical transmembrane domain (TM), and phase separation showed that it is an integral membrane protein. In AcMNPV-infected cells, Ac76 was detected as a stable dimer that was resistant to SDS and thermal denaturation, and only a trace amount of monomer was detected. A co-immunoprecipitation assay demonstrated the dimerization of Ac76 by high-affinity self-association. Covalent cross-linking results showed that higher-order oligomers of trimer, tetramer, hexamer and octamer as well as the stable dimer were detected in virus-infected cells. Bioinformatic analysis suggested that the leucine- and isoleucine-rich sequence in the TM helix of Ac76 likely forms a leucine/isoleucine zipper to mediate the helix-helix interaction of Ac76 with itself. A recombinant virus in which L^{26}, L^{29} and L^{33} in the TM of Ac76 were all substituted with alanines was constructed. Analysis of the mutant revealed that the leucines in the TM of Ac76 are important for infectious BV production and normal-appearing intranuclear microvesicle formation.

Contributed paper. Thursday, 15:30. **249**

High-throughput purification of dsRNA against sacbrood virus disease in honey bees *Apis cerana* (Hymenoptera: Apidae)

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The importance of honey bees to the world economy does not hang on bee products, but pollination of 80% food crops. However, like other animals, honeybee is inevitably subject to infection by a wide variety of pathogens that are responsible for significant colony losses. Sacbrood virus (SBV) is a serious hazard disease to honey bees (*Apis cerana*). Relying heavily on chemical agent for the control of this disease, problems of resistance and pollution are perplexing beekeeping. Therefore, beekeeping calls for environmentally friendly technology of disease management, especially the antiviral bee breeding. Using RNA interference technology is a cost-effective approach for disease bio-control. To address this issue, large-scale and pure dsRNA is in great need. A length of 699 bps *Vp1* gene of SBV was selected to be expressed with L4440 plasmid in *Escherichia coli* HT115 (DE3). After ultrasonic disruption and ethanol precipitation, *Vp1*-dsRNA molecules were purified with anion exchange chromatography utilizing convective interaction media (CIM) monolithic columns. RNAi was performed to prevent bees from SBV under laboratory conditions. Comparing with bees without dsRNA, *Vp1*-dsRNA prevented 49% to 75% larval mortality of *A. cerana* from SBV infection. The result may provide a model in large-scale use of RNAi for SBV control.

Funding: This work was supported by National Natural Science Foundation of China (No: 31301924), Natural Science Foundation of Guangdong Province
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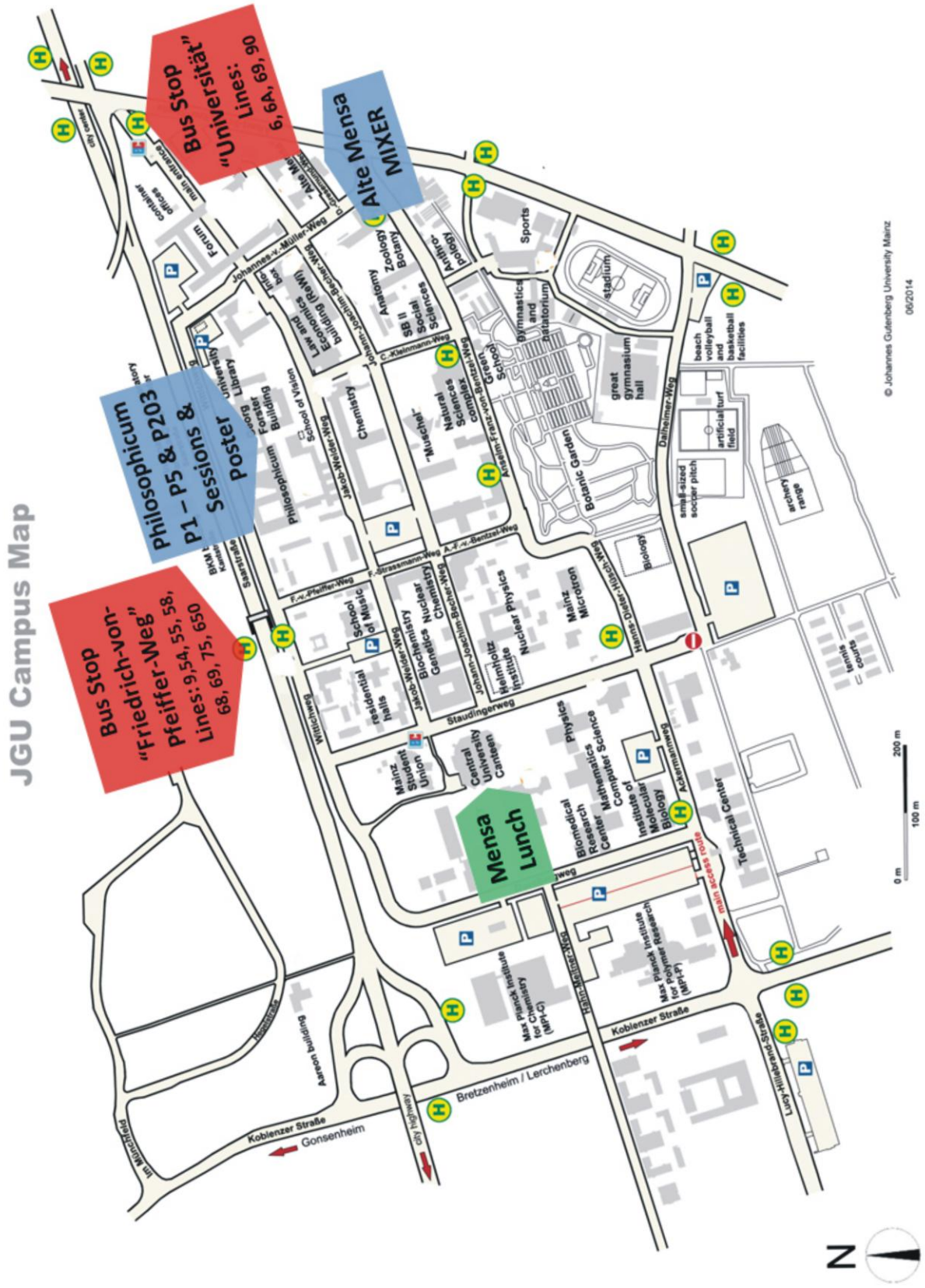
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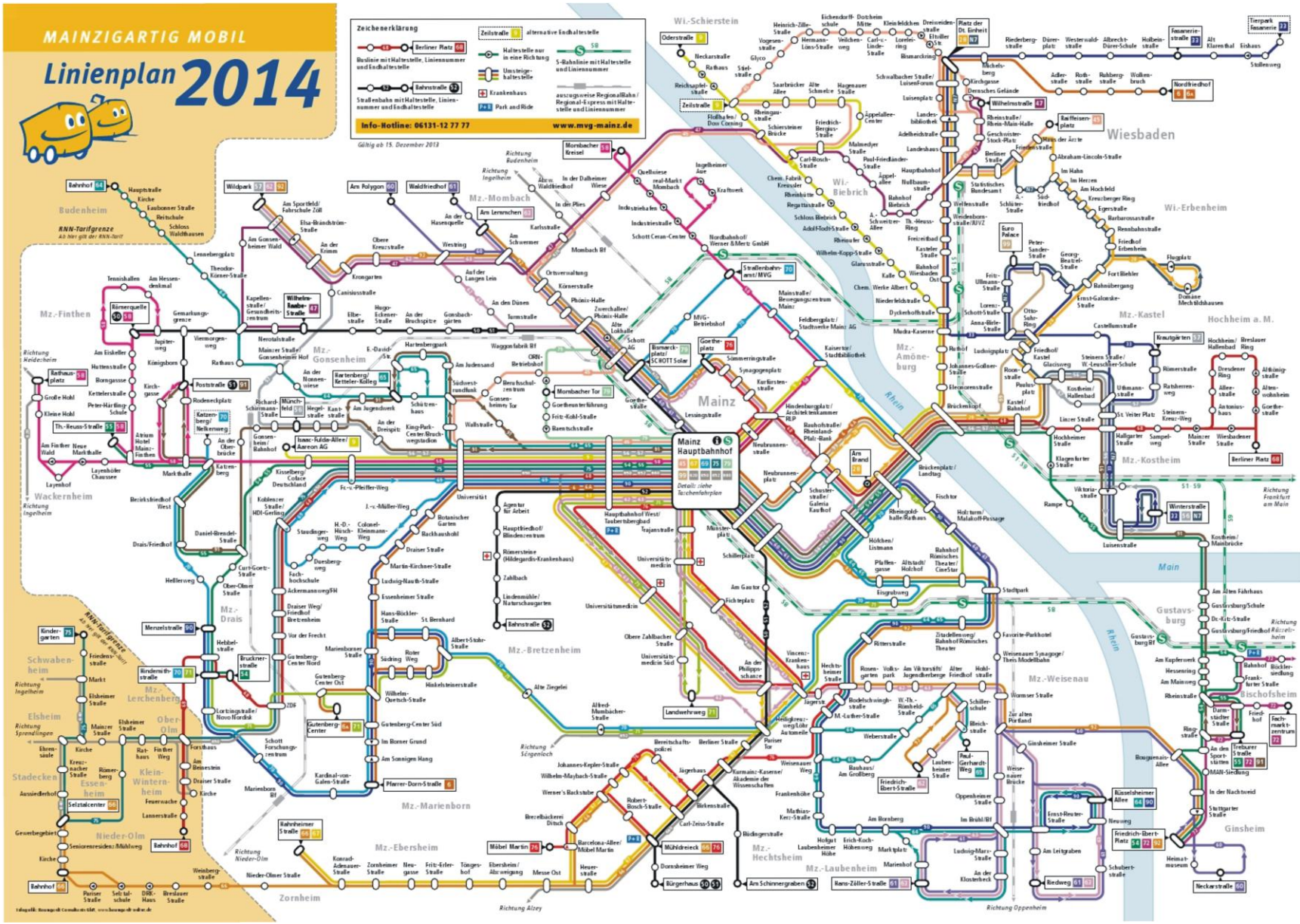
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Wednesday - 6 August

7:30-18:00	Registration	P1
8:00-10:00	***Symposium 5 (Microbial Control Division)***	P3
	Developments/Issues in the Regulation of Microbial Products: Harmonization across Jurisdictions	
	The authorisation and regulation of microbial biopesticides: why bother? *David Chandler*	
	Registration of Biopesticides in the EU: a company perspective *Philip Kessler*	
	Biopesticide registration, a company perspective and how registration influences biopesticide R&D approach of companies in North American *Jarrod Leland*	
	Registration of biopesticides: how research can be structured to suit microbial registration needs and promote the commercial development of new biopesticides *Roma Gwynn*	
	Current developments and issues on regulation of biopesticides- Lessons from REBECA project, comparison of EU and USA systems *Sabine Asser-Kaiser*	
8:00-10:00	**Contributed Papers**	
	Bacteria 3	P5
	Diseases of Beneficial Invertebrates 2	P4
	Fungi 4	P2
10:00-10:30	Break	
10:30-12:30	***Symposium 6 (Bacteria Division)***	P5
	Structure and Function of Novel Insecticidal Toxins	
	Structural and biophysical characterization of Cry34Ab1 and Cry35Ab1 *Matthew S. Kelker*	
	Structure/function studies of Cry5B via alanine-scanning mutagenesis *Raffi V. Aroian*	
	Insights into the structures of non-3-domain toxins through structural modelling *Colin Berry*	
	Novel MTX Toxins for Insect Control *Yong Yin*	
	Insecticidal toxins from Photorhabdus luminescens and asymbiotica, targeting the actin cytoskeleton and GTP-binding proteins *Thomas Jank*	
	Molecular basis of parasporin-2 action toward cancer cells *Sakae Kitada*	
10:30-12:30	**Contributed Papers**	
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	Viruses 4	P1
	Fungi 5	P2
12:30-14:00	Lunch	Mensa
12:30-14:00	**Student Workshop + Pizza**	P2
	R. Humber, M. Goettel, Y. Inoue	
	HOW TO WRITE A PAPER	
13:15-14:00	JIP Editorial Board Meeting	P203
14:00-16:00	**Contributed Papers**	
	Microsporidia 1	P4
	Microbial Control 3	P3
	Viruses 5	P1
	Bacteria 4	P5
16:00-16:30	Break	
16:30-18:30	**Poster Session**	Philosophicum
	All Divisions	
20:00-21:30	**Division Business Meetings and Workshops**	
	Bacteria	P5
	Microsporidia	P4
	Fungi	P2
	Viruses	P3

Thursday - 7 August

7:30-16:30	Registration	P1
8:00-10:00	***Symposium 7 (Diseases of Beneficial Invertebrates)***	P2
	Emerging Tools for Aquatic Pathogen Discovery and Description	
	Early mortality syndrome is an infectious disease with a bacterial etiology *Loc Tran*	
	Policy, phylogeny, and the parasite *Grant D. Stentiford*	
	The Next Generation of Crustacean Health: Disease Diagnostics Using Modern *Transcriptomics K. Fraser Clark*	
	Environmental DNA as a tool for detection and identification of aquatic parasites: known unknowns and just plain unknowns *Hanna Hartikainen*	
8:00-10:00	**Symposium (DFG Priority Program)**	P5
	Organizer: Joachim Kurtz	
	Host Parasite Coevolution	
8:00-10:00	**Contributed Papers**	
	Nematodes 3	P4
	Viruses 6	P1
10:00-10:30	Break	
10:30-12:30	**SIP Annual Business Meeting**	P1
	Presiding: Jørgen Eilenberg	
12:30-14:00	Lunch	Mensa
14:00-16:00	***Symposium 8 (Cross-Divisional)***	P2
	Host-Pathogen Ecology at the Molecular Level: Gene Regulation and Environment Sensing	
	The *Bacillus thuringiensis* way of life: communicate to kill and survive in the insect host *Didier Lereclus*	
	The interplay of Paenibacillus larvae with honey larvae during infection *Elke Genersch*	
	Antimicrobial defense and persistent infection in insects revisited *Jens Rolff*	
	Vibrio and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes *Delphine Destoumieux-Garzón*	
14:00-16:00	**Contributed Papers**	
	Microbial Control 4	P3
	Viruses 7	P1
16:00-16:30	**Student Business Meeting**	P4
18:30	Departure from Hotels to Banquet	
19:00-1:00	Reception and Banquet	Alte Lokhalle

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