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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### Sunday – 3 August

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<td>Potentials for utilizing and controlling insect pathogens&lt;br&gt;Richou Han</td>
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<td>Story of an African firm: 10 years in the biopesticide business – lessons learned along the way&lt;br&gt;Sean Moore</td>
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<td>A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods&lt;br&gt;Willem J. Ravensberg</td>
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<td>BASF Functional Crop Care. Unlocking Agricultural Potential in Soil, Seed and Crop&lt;br&gt;Sebastian Bachem</td>
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<td>Small molecule signals in nematodes - common motifs and species specific modifications&lt;br&gt;Stephan H. von Reuss</td>
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<td>Olfactory Plasticity in Entomopathogenic Nematodes&lt;br&gt;Elissa Hallem</td>
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<td>Multiple Consequences of Belowground Herbivore Induced Volatile Signals&lt;br&gt;Jared G. Ali</td>
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<td>Root Zone Chemical Ecology; New Techniques for Below Ground Sampling and Analyses of Volatile Semochemicals&lt;br&gt;Hans T. Alborn</td>
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<td>A new intracellular parasite is a missing link between fungi and microsporidia&lt;br&gt;Karen L. Haag</td>
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<td>Parasite takes fly - A Drosophila model of Microsporidia infection&lt;br&gt;Sebastian Niehaus</td>
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<td>Nematodes</td>
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<td>7:30-13:00</td>
<td>Registration</td>
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<td>8:00-10:00</td>
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<td>Fusarium: Host-Plant Interactions and the Mycotic Universe&lt;br&gt;Kevin M. Myles</td>
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<td>Fusarium and its Relation to Insect Pest Control&lt;br&gt;Raul Andino</td>
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<td>How Fusarium graminearum influences insect-plant interactions&lt;br&gt;Tao Zhao</td>
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<td>Fusarium graminearum and the rice Blast fungus&lt;br&gt;A. J. Jackson</td>
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<td>Controlling fungal, bacterial and viral plant pathogens&lt;br&gt;Drakulic Jassy</td>
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<td>Plant-microorganism interactions that shape host-plant selection in the grapevine moth&lt;br&gt;Marco Tassin</td>
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<td>Effect of host plant on aphid susceptibility to the fungal pathogen Pandora neoaphidis&lt;br&gt;Cezary Tkaczuk</td>
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<td>Nematodes 2</td>
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<td>Role of cellular and virus-encoded microRNAs in insect-host-virus interactions&lt;br&gt;Sassan Asgari</td>
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<td>Sensing viral RNA in Drosophila melanogaster&lt;br&gt;Carine Meignin</td>
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<td>Caratinae Meignin Small RNA-directed antiviral immunity in disease-vector mosquitoes&lt;br&gt;Kevin M. Myles</td>
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<td>Controlling viral infections in insects&lt;br&gt;Raul Andino</td>
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<td>15:00</td>
<td>Departure of buses for BBQ + 5K</td>
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of the
SOCIETY FOR
INVERTEBRATE
PATHOLOGY
and
INTERNATIONAL CONGRESS ON
INVERTEBRATE PATHOLOGY
AND MICROBIAL CONTROL
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INVERTEBRATE PATHOLOGY
AND MICROBIAL CONTROL

PROGRAM and ABSTRACTS

3-7 August 2014
University of Mainz, Germany
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  - **Yu-Lin Gao**, Member-at-Large

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  - **Johnny Shajahan**, Member-at-Large  
  - **Thomas Steele**, Student Representative

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  - **Ingeborg Klingen**, Vice-Chair/Chair-Elect  
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  - **Tarryn Goble**, Member-at-Large  
  - **Chad Keyser**, Student Representative  
  - **Joanna Fisher**, Student Representative

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  - **Glen Stevens**, Secretary/Treasurer  
  - **Luis Leite**, Member-at-Large  
  - **Fatma Kaplan**, Member-at-Large  
  - **John G. McMullen**, Student Representative
Division of Microbial Control
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Michael Brownbridge, Secretary/Treasurer
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Jarrod Leland, Member-at-Large
Patricia Golo, Student Representative

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Martin Erlandson, Vice-Chair/Chair-Elect
Rollie Clem, Secretary/Treasurer
Bob Harrison, Member-at-Large
Elisabeth Herniou, Member-at-Large
Aurélien Chateigner, Student Representative
Yasumasa Saito, Student Representative

Division of Diseases of Beneficial Invertebrates
Elke Genersch, Chair
Hamish Small, Vice-Chair/Chair-Elect
Kate Aronstein, Secretary/Treasurer
Eva Garcia-Gonzalez, Member-at-Large
Gillian Hertlein, Student Representative

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Lee Solter (Chair)
Mark Goettel
Harry Kaya
Madoka Nakai
Just Vlak

Meetings
Nina Jenkins (Chair)
Kelli Hoover
Zhizong (Rose) Hu
Johannes Jehle
Jean-Louis Schwartz
Brian Federici (ex off.)

Publications
David Shapiro Ilan (Chair)
Albrecht Koppenhöfer
Bryony Bonning
Selcuk Hazir
Jørgen Eilenberg (ex off.)
Eric Haas-Stapleton (ex off.)
Brian Federici (ex off.)
Leellen Solter (ex off.)
Cecilia Schmitt (ex off.)

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Nina Jenkins (Chair)
Robert Anderson
Steven Arthurs
Sassan Asgari
Susan Bornstein-Forst
Sunday Ekesi
Carrie Hauxwell
Kelli Hoover
Annette Jensen
Yasuhsa Kunimi
Nicolai Meyling
Helen Roy
Jean-Louis Schwartz
Steven Valles

Endowment & Financial Support
Roma Gwynn (Chair)
Michael Brownbridge
Michael Dimock
James Harper
Dirk Ave
Kelli Hoover (ex off.)

Founder’s Lecture
James Becnel (Chair)
Neil Crickmore
Zhihong (Rose) Hu
Harry Kaya

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Siva Jakka (Bacteria Division)
n.n. (Fungi Division)
Siva Jakka (Microbial Control Division)
Thomas Steele (Microsporidia Division)
John McMullen II (Nematode Division)
Jörg Wennman (Virus Division)

History
Elizbeth Davidson (Chair)
James Harper
Don Roberts
Harry Kaya
Fernando Vega
Jürg Huber
Mark Goettel

Awards & Student Contest
Monique van Oers (Virus Division and DBI; Chair in 2014)
Suendra Dara (Microbial Control Division)
Hyun-Woo Park (Bacteria Division)
Andreas Linde (Microsporidia Division)
Patricia Stock (Nematode Division)
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Co-Chair: Thomas Hankeln
Program: Elke Genersch, Andreas Linde
Secretary: Regina Kleespies
Treasurers: Katharina Saar, Dietrich Stephan
Meeting’s Web site: Gianpiero Gueli Alletti,
Local Arrangements and registration software: Eva Fritsch, Manuela Gebhardt, Andreas Larem, Juliana Pelz, Annette Sauer, Diana Schneider, Hadil Sharifova, Karin Undorf-Spahn, Jörg Wennmann
5K Race: Thomas Guthmann
Miscellaneous assistance: Sylvia Adamek, Tanja Bernhardt, Carina Ehrich, Jaqueline Franck, Susanne Ganzer, Gudrun Knuff, Christina Matecki, Laurin Monnheimer, Christopher Seib, Zachary Taylor
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PROGRAM
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

Opening Ceremony and SIP Founders’ Memorial Lecture

Opening Ceremonies
Johannes Jehle, Chair, Organizing Committee
Jørgen Ellenberg, 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 Bichou Han, Xuehong Qiu and Xin 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, Berken 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 Volatiles Semiochemicals Hans T. Alborn1, Fatna Kaplan2; 1USDA ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, U.S.A.; 2Kaplan Schiller Research LLC and Biology Dept. University of Florida, 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. Alborn1, Fatna Kaplan2; 1USDA ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, U.S.A.; 2Kaplan 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 Yalamp1; Dan Altier1, Jennifer Barry1, Jarred Oral1, Ute Schellenberger1, Adane Negatu1, Scott Diehn1, Virginia Crane1, Gary Sandahl1, Joe Zhao1; Dave Cerf2, Claudia Perez Ortega1, Mark Nelson1, Analiza Alves1, Lu Liu1, Gusui Wu1; DuPont Pioneer, Johnston, IA, U.S.A.; 2DuPont Pioneer, Hayward, CA, U.S.A.; 3DuPont, 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 (Lepidoptera: Tortricidae) pupae Nurcan Albayrak Iskender1, Ya’par Aksu2; 1Artvin Coruh University, Faculty of Arts and Sciences, Department of Biology, Artvin, Turkey; 2Artvin 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 Kupferschmidt¹, Maria Pechy-Tarr¹, Nicola Imperiali¹, Monika Maurhofer¹, Christoph Keel¹, Department of Fundamental Microbiology, University of Lausanne, Switzerland; ²Plant 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 Hertlein¹; Sebastian Müller²; Eva Garcia-Gonzalez²; Roderich D. Süssmuth²; Elke Genersch¹,²; ¹Institute for Bee Research Hohen Neuendorf, Germany; ²Technische Universität Berlin, Institut für Chemie, Berlin, Germany; ³Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany

14:15 **14 Two new Bacillus thuringiensis toxins active against Lepidoptera and Coleoptera** Mikel Domínguez¹, Héctor Ruiz de Escudero²,³; Isabel Matas³; Leopoldo Palma³; Delia Muñoz³, Primitivo Caballero³, ¹Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Multyva, Spain; ²Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, Pamplona, Spain

15:30 **15-STU** Entomopathogenic *Bacillus thuringiensis* as PGPR Jiaheling Qi¹,²; Daigo Aiuchi²; Shin-ichiro Asano²; Masanori Koike³, ¹The United Graduate School of Agricultural Sciences, Iwate University, Iwate Japan; ²Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan; ³Department 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-Joven¹, Jean-Luc Rolland¹, Eric Abaddé¹, Mohamed Laabir¹, Estelle Masserot¹, Audrey Vanhove¹, Audrey Caro¹, Delphine Bonnet¹, Delphine Destoumieux-Garzon¹, ¹Ecolegy of coastal marine systems, UMR 5119, CNRS, Ifremer, IRD, University of Montpellier, France; ²Laboratoire Environnement Ressource du Languedoc Roussillon, Ifremer, Sète, France.

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 Gasmì, Agata K. Jakubowska, Juan Ferré, Salvador Herrera; Laboratory of Biochemical Genetics and Biotechnology, Department of Genetics, Universitat de València 46100 – Burjassot (València), Spain

14:45 **20 Entry of Bombyx mori nucleopolyhedrovirus (BmNPV) into BmN Cells by Macropinocytic Endocytosis** Jinshan Huang¹,²; Bifang Hao¹,²; Chen Cheng¹; Fei Liang¹, Xingjia Shen²,³; ¹Sericultural Research Institute, Jiangsu University of Science and Technology, ²Sericultural Research Institute, Chinese Academy of Agricultural Science, Zhenjiang, Jiangsu, PRC, China

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

15:15 **22 Nuclear localization and other domains of Autographa californica nucleopolyhedrovirus DNA polymerase Guozhong Feng¹; Peter Krell²; ¹State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, 310006, China; ²University of Guelph, Guelph Ontario, Canada

15:30 **23 STU** Investigations into the role of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) AC141 (EXON0) and *Trichoprosopina ni* kinesin-1 in budded virus nucleocapsid egress Siddhartha Biswas¹; Gary W. Blissard²; David A. Theilmann³, ¹Plant Science, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC Canada; ²Boyce Thompson Institute at Cornell University, Ithaca, NY, USA; ³Pacific 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 S. Ausive Department of Entomology and Acaology, ESALQ, University of São Paulo, Piracicaba, São Paulo, Brazil

14:15 **26 Effectiveness of biorationals and *B. bassiana* against tomato fruitworm in Sinaloa** Cipriano Garcia, 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 Goble¹,²; Ann Hajek³; Mark Jackson⁴; and Sana Gardesco²; ¹Department of Entomology, Cornell University, Ithaca, USA; ²USDA-ARS-NCAUR, Crop Bioprotection Research Unit, Peoria, IL, USA
16:45  28 STU Management of entomopathogenic fungal disease in rearing mealworms, *Tenebrio molitor* as animal feed  Siyeon 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 Karanja1,2, Ronny Larsson1,2, Tobias M. M. Asmamaw Teshome1,2, Marie Fesselet1,2, Vladimir Půža1,2, Fabio Mascher1,2, Ted C.J. Turlings1,2, 1FarCARE Laboratory, University of Neuchâtel, Neuchâtel (Switzerland); 2Laboratory of Entomopathogenic Nematodes, Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

15:15  30 Management of *Frankliniella occidentalis* (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi  Jeane Su Kim1,2,3,4,5, 1National Applied Research Laboratories (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi; 2Department of Agriculture and Natural Resources, Louisiana State University, Baton Rouge LA, USA; 3Institute of Cytology, St. Petersburg, Russia; 4Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL 32608, USA; 5Department of Pathology, Division of Parasitology and Tropical Medicine, and Department of Medicine Division of Infectious Diseases, Albert Einstein College of Medicine, Bronx, NY, USA

16:00–16:30 BREAK

Symposium 2 (Microsporida)  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. Haag1, Timothy Y. James1, Ronny Larsson2,3,4,5, Tobias M. M. Schaer2,3, Dominik Refardt2,4,6, Dieter Ebert2, 1Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil; 2University of Michigan, Ann Arbor, MI, USA; 3University of Lund, Lund, Sweden; 4Basel University, Basel, Switzerland; 5Zurich University of Applied Sciences, Campus Grüental, Wädenswil, Switzerland

17:00  32 Parasite takes fly - A Drosophila model of Microsporidia infection  Sebastian Niehus1,2, Adrien Franchet1, Frédéric Delbac1, Michael Boutros1, Dominique Ferrandon1, 1Institut de Biologie Moléculaire et Cellulaire, UPR 9022 du CNRS, Université de Strasbourg, Strasbourg, France; 2Laboratoire Microorganismes: Génome et Environnement, UMR 6023 du CNRS, Université Blaise Pascal, Aubière, France; 3German 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  Yuuya Y. Sokolova1,2,3, Core Microscopy Center, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, USA; 2Institute of Cytology, St. Petersburg, Russia.

18:00  34 Microsporida: Pathogens of Opportunity  James J. Becnel1, Louis M. Weiss2; 1Center for Medical, Agriculture and Veterinary Entomology, USDA/ARS, Gainesville, FL 32608, USA; 2Department 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-Herrera1,2,3,4, Geoffrey Jaffuel1,2, Xavier Chiriboga1,2, Rubén Blanco-Pérez1,2, Marie Fesselet3, Vladimir Půža1,2, Fabio Mascher1,2, Ted C.J. Turlings1,2; 1FarCARE Laboratory, University of Neuchâtel, Neuchâtel (Switzerland); 2Département fédéral de l'économie, de la formation et de la recherche DEFR, Agroscope, Institut des Sciences en Production Végétale IPV, Nyon (Switzerland); 3Laboratory 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*  Annette Zener, 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 Addis1,2,3, Asmamaw Teshome1,2, Olaf Strauch1 and Raif-Udo Ehlers1; 1Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University, Kiel, Germany; 2Department of Biology, Ghent University, Ghent, Belgium; 3Genevoss, GmbH, Schwenental, Germany

17:30  39 STU How does plant domestication influence entomopathogenic nematodes as potential biological control agents?  Monique Rivera1, Cesar Rodriguez-Saona1, Hans T. Alborn1, and Albrecht M. Koppenhöfer1; 1Department of Entomology, Rutgers University, New Brunswick, NJ 08901, USA, 2USDA ARS GMAVE, Gainesville, FL, USA

17:45  40 Analysis of intraspecific variability in *Steinernema kraussei* populations using PCA, M. Clausi1,2,3, G. Rappazzo1, E. Tarasco1, D. Leone1, M. T. Vinicuerga1; 1Department of Biological, Geological and Environmental Sciences, Section of Animal Biology “M. La Greca”, University of Catania, Catania (Italy); 2Department 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 entomopathogenic nematode *Steinernema affine* (Steinernematidae: Nematoda) inferred using microsatellite markers  Vladimir Půža1,2,3, Martina Žurovcová1, Jiří Nemut1, Daniela Chundelová1,2, Zdeněk Mráček1; 1Institute of Entomology, Biology Centre of the AS CR, České Budějovice, Czech Republic; 2Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic
18:15 **STU Eat or Be Eaten: Fungus and Nematode Switch off as Predator and Prey** E. Erin Morris and Ann E. Hajek, 1 Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg 1871, Denmark; 2Department of Entomology, Cornell University, Ithaca, New York 14853-2601, USA

18:15 **STU 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

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

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

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

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

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

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

18:00 **STU The effects of temperature on *Cryptophlebia leucotreta* granulovirus (GrleGV-SA) in mortality rates of false codling moth larvae Thaumatomadia leucotreta Devon Brits, Jaryd Ridgway & Alicia Timm, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa
TUESDAY - 5 August

07:30-13:00 Registration

Symposium 3 (Fungi) Tuesday, 8:00-10:00.
Fatal Attraction: Fungi and Odours in deadly Combinations for Pest Control
Organizer/Moderator: Ingeborg Klingen

8:00 58 Conifer - bark beetle - fungus interactions Tao Zhao¹, Paul Kroken², Anna-Karin Borg-Karlson¹, The Royal Institute of Technology, Department of Chemistry, Ecological Chemistry Group, Stockholm, Sweden; ¹Norwegian 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 Schumann¹, Anant Patil¹, Miriam Hanitzsch¹, Stefan Vidal¹, Georg-August-Universität Göttingen, Department of Crop Sciences, Göttingen, Germany; ²Fachhochschule 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 Jacobsen¹, Jørgen Eilenberg¹, Edmond Mach Foundation, 38010 San Michele all’Adige, Italy; ²Integrated Plant Protection, Dep. of Crop Protection Biology, Swedish University of Agricultural Sciences, Sweden
9:00 61 STU How Fusarium graminearum influences insect-plant interactions Drakulic Jassy¹; Bruce Toby², Ray Rumiana², ¹Division of Plant and Crop Sciences, University of Nottingham, UK; ²Rosatom, 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. Knudsen¹, Ilaria Pertot², Marco Tasin¹,², ¹Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1422 Ås, Norway; ²Edmund Mach Foundation, 38010 San Michele all’Adige, Italy
9:40 63 Effect of host plant on aphid susceptibility to the fungal pathogen Pandora neoaphidis Cezary Tkaczk¹; Paresh A. Shah¹, Judith K. Pell¹,², ¹Department of Plant Protection, Siedlce University, Siedlce, Poland; ²Plant and Invertebrate Ecology Department (now AgroEcology Department), Rothamsted Research, Harpenden, UK; ³Current Address: J.K. Pell Consulting, Luton, UK

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

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. Stasko, Molecular Parasitology Group, Genome Biology Division, New England Biolabs, 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 Anaís Castagnola¹; Nathaniel Davis¹; Belen Molina¹; S. Patricia Stock¹; John G. McMullen II²; ¹Department of Entomology, University of Arizona; ²Center for Insect Science, University of Arizona; ³Pima Community College; ⁴Department 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 (Enterobacteriaceae), John G McMullen II¹; Gaelle Bisch²; Jean-Claude Ogier³; Sylvie Pagès²; Sophie Gaudriault²; S. Patricia Stock¹; ¹University of Arizona, School of Animal and Comparative Biomedical Sciences, Tucson, AZ; ²Université Montpellier II/INRA, UMR 1333 Laboratoire DGIM, Montpellier, France; ³University of Arizona, Department of Entomology, Tucson, AZ, USA
9:00 68 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 Biowissenschaften, Goethe Universität Frankfurt, Germany
**VIRUSES 3**

**Contributed Papers**

Moderators: Zhihong Hu and Trevor Williams

**Monday, 08:00-10:00.**

*9:00* 71 Characterization and formulation of a Colombian isolate of *Eriovixia ello granulovirus* (L.) (Lepidoptera: Sphingidae) Juliana Gómez1, Gloria Barrera1, Paola Cuartas1, Carolina Ruiz1, Adriana Santos1, Liz Uribe1, Guillermo León2, Laura Villamizar1; 1Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia, 2Centro de Investigación “La Libertad” Corpoica, Puerto López, Colombia

*9:15* 72 PRODUCTION OF the *Cydia pomonella* granulovirus (CpGV) IN A HETEROLOGOUS HOST. C.B. Chambers1, S.D. Moore1, 2, M.P. Hill2 & C. Knox1, 1River Bioscience, PO Box 20398, Humewood 6013, Port Elizabeth, South Africa, 2Citrus Research International, PO Box 20285, Humewood 6013, Port Elizabeth, South Africa.

*9:30* 73 Post-translational cleavage of P74 of the Helicoverpa armigera single nucleopolyhedrovirus facilitates per os infection Huachao Huang1, Manli Wang1, Xin Luo1, Xi Wang1, Bassi M. Afri2, Fei Deng2, Hualin Wang1, Zhihong Hu1; 1State Key Laboratory of Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, PR China; 2Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

*8:30* 74-STU Isolation, genetic characterisation and evaluation of biological activity of a novel South African *Phthorimaea operculella* granulovirus (PhopGV) Michael D. Juokes1, Caroline M. Knox1, Sean D. Moore1, Martin P. Hill2, 1Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140 South Africa, 2Citrus Research International, Humewood, Port Elizabeth, 6013 South Africa.

*8:45* 75 Genetic and biological characterisation of a novel South African *Plutella xylostella* xylella granulovirus, PtxyGV-SA Fatima Abdulkadir1, Caroline Knox1, Tamryn Marsberg1, Martin P. Hill2, 1Department of Biochemistry, Microbiology and Bacteriology, Rhodes University, Grahamstown, 6140 South Africa, 2Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa.

**BACTERIA 2**

**Contributed Papers**

Moderators: Jean-Louis Schwartz and Juan Ferré

**Monday, 08:00-10:00.**

*9:00* 79 *Yersinia enterocolitica* MH96 (Enterobacteriaceae) BC subcomplex of the *Yen-Tc AB* toxin is able to induce toxicity independent of the *A* subcomplex Sean D.G. Marshall1, Jason N. Busby2, J. Shaun Lott3, Sandra A. Jones1, Julie E. Dalziel1, Femke Schepers4, Mark Hurst1; 1Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch 8140, New Zealand; 2School of Biological Sciences, University of Auckland, New Zealand; 3Food & Bio-based Products, AgResearch, Grasslands Research Centre, Palmerston North 4442, New Zealand

*9:15* 80 Interaction of *Bacillus thuringiensis* Cry1Ab toxin with Mucus-rich structures Diego Segond1,2, Agnès Rejasse1, Christophe Buisson1, Shuyuan Guo1,2,3, Karine Adel-Patient1,2, Hervé Bernard2,3, Didier Lereclus1, Christiina Nielsen-LeRoux3; 1INRA UMR1319-Micalis, team GME, 78352 Jouy en Josas France, 2INRA, UR496 Unité d’Immuno-Allergie Alimentaire, France, 3School of Life Science, Beijing Institute of Technology, Beijing, China, 4CEA, IBITecS, Service de Pharmacologie et d’Immunanalyse, Gif-sur-Yvette, France

*9:30* 81-STU Pore formation helping ability and binding affinity of BmABCC2 and Bir175 against Cry1A toxins Shihho Tanaka1, Ami Iizuka1, Kazuhiwa Miyamoto2, Hiroaki Noda2, Shingo Kikuta3, Ryoichi Sato1; 1Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan; 2National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan

*8:45* 82 A necessary step in the mode of action of the Cry6 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? Alícia Elhigazi, Vidisha Krishnan, Fatali 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 Narvaez1, Vincent Vachon2, Dong Xu3, Jean-Charles Côté4, Jean-Louis Schwartz4; 1Groupe d’étude des protéines membranaires, Université de Montréal, Montréal, Quebec, Canada; 2Research Center, Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada, 3Centre 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 Latsenko, Iuliia Boichenko, Ralf J. Sommer; Max Planck Institute for Developmental Biology, Department for Evolutionary Biology, Tuebingen, Germany

**10:00–10:30** 
**BREAK**

**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 Paro1,2, Eric Aguiera1, Bill Claydon1, Jose Trindade Marques1, Jean-Luc Imler1,2 and Carine Meignin1,2; 1IBMC, CNRS-UPR9022, Strasbourg, France; 2Laboratory of RNA Interference, Biochemistry and Immunology, Universidade Federal de Minas Gerais Belo Horizonte, Brazil; 3University 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 Paul 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 biocoms, 
Naworaj Acharya1, Simon Blanford2,3, Edwin G. Rajotte2, Nina E. Jenkins1, Mathew B. Thomas1,2; 1Department of Entomology, Penn State University, 501 Agricultural Sciences and Industries Building, PA 16802, USA; 2Center 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. A. Chea-ma1, Eric W. Cornelius1, Vincent Y. Eziah1, Ken O. Fening1, Clare Storm2, Dave Moore3, Nick Jessop3, Matthew Smith4, Olivier Potin1, Pierre Grammaire5 and Belinda Luke6; 1Department of Crop Science, University of Ghana, Legon; 2Exosett Ltd, UK; 3CABI, UK; 4SylvanBio, France

**11:15** 
**94-STU** Lack of involvement of chitinase in direct toxicity of *Beauveria bassiana* exudates to the aphid *Myzus persicae* 
Peter Cheong1, Travis R. Glare1, Michael Rostas1, Stephen Haines2, Jolon Dyer2, Stefan Cierrens3, Jenny Brookes1 and Stephen Ford1; 1Bio-Protection Research Centre, P O Box 85084, Lincoln University, Lincoln 7647, Christchurch, New Zealand; 2AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand; 3Biotelluga Limited, Pukekohe 2120, New Zealand

**11:30** 
**95-STU** Entomopathogenic fungi for control of false codling moth in South African citrus orchards 
Candice A. Coombes1; Martin P. Hill1; Sean D. Moore1,2; Joanna F. Dames1,2; Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140, South Africa; 3Citrus Research International, Humewood, 6013, Port Elizabeth, South Africa; 4Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140, South Africa

**11:45** 
**97-STU** Wireworm control with entomopathogenic fungi and plant extracts 
Sonja Eckard1; Sven Bacher2; Jürg Enkerli1; Giselaer Grabenweger2; Agroscope, Institute for Sustainability Sciences, Reckenholzstrasse 191, Zürich, Switzerland; 2University of Fribourg, Department of Biology, Unit of Ecology and Evolution, Fribourg, Switzerland

**12:00** 
**98-STU** Long-term persistence of *Beauveria bronniarti* 2 used for cockchafer control in the Euroregion Tyrol 
Johanna Mayerhofer1,2; Jürg Enkerli1; Roland Zeller2 and Hermann Strasser1; 1Institute of Microbiology, Leopold-Franzens University Innsbruck, AUT; 2Molecular Ecology, Institute for Sustainability Sciences, Agroscope, Zürich, CH; 3Research Centre for Agriculture and Forestry Laimburg, Ora/Auer, Italy

**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. Behringer1,2, Mark J. Butler IV3, Jessica Moss1, Jeffrey D. Shields3; 1University of Florida, Program in Fisheries and Aquatic Sciences, Gainesville, Florida 32653 (USA); 2University of Florida, Emerging Pathogens Institute, Gainesville, Florida 32611 (USA); 3Old Dominion University, Department of Biological Sciences, Norfolk, Virginia 23529 (USA); 4Virginia 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 *Anaphtheroides haemophilia*, Spencer J. Greenwood1,2; K. Fraser Clark1,2,3; Atlantic Veterinary College Lobster Science Centre; 1Department of Biomedical Sciences, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada; 2Department of Plant and Animal Sciences, Dalhousie University, Truro, Nova Scotia, Canada

**11:00** 
**101-STU** Metabolic investigation of Bitter Crab Disease in snow crabs (*Chionoecetes opilio*) 
Melanie
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 Truquet1, Heike Feldhaar2, J. J. See Pedersen3; Animal Ecology I, University of Bayreuth, Germany, 2Centre 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 Amtt Wynn1, Annette Bruun Jensen1; Celeste d’Allesandro2, Jørgen Eilenberg3; 1Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; 2Department of Entomology and Acracology, ESALQ, University of São Paulo, Brazil

12:00 114 Effects of entomopathogenic fungi on the Trialeurodes vaporariorum – Encarsia formosa system: preliminary results Monica Oreste, Eugenio 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

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 Chandler1, Liam Harvey & Wyn Grant2; 1Warwick Crop Centre, School of Life Sciences, University of Warwick, UK; 2Department 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
8:00  120 Resistance alleles to Lysinibacillus sphaericus are co-select in a Culex quinquefasciatus colony and display distinct features Maria Helena N. L. Silva-Filha, Karlos D. M. Chaleg, Tatiana P. Romão, Daniella A. Tavares, Hervely S. G. Menezes, Cláudia M. F. de Oliveira, Osvaldo P. de-Melo-Neto, Department of Entomology, Department of Microbiology, Centro de Pesquisas Aggeu Magalhães-FIOCRUZ, Recife, Brazil

8:15  121-STU Untangling insect pathogenicity in plant-beneficial pseudomonads on a combination of comparative genomics, bioassays and histopathology Pascale Flury, Beat Ruffner, Shakra Fataar, Maria Péchy-Tarr, Regina G. Kleespies, Cornelia Ullrich, Johannes A. Jehle, Theo H. M. Smits, Christoph Keel, Monika Mauroher, Institute of Plant Pathology, Swiss Federal Institute of Technology, Zürich, Switzerland; Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; 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 Ligia M. Ferreira, Nathaly A. do Nascimento, Tatiana P. Romão, Antônio M. Rezende, Osvaldo P. de-Melo-Neto, Maria Helena N. L. Silva-Filha, Department of Entomology, 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 Lestredat, 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, Fred Gould, David G. Heckel, Clemson University, Clemson, South Carolina, USA; North Carolina State University, Raleigh, North Carolina, USA; 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, Xue Chang, Anghard M. R. Gatehouse, Zhenying Wang, Martin E. Edward, Kanglai He, The State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China; Newcastle Institute for Research on Environment and Sustainability, School of Biology, University of Newcastle, UK

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

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

BACTERIA 3

Moderators: Juan Luis Jurat-Fuentes and David Heckel

8:00  117 Biopesticide registration, a company perspective and how registration influences biopesticide R&D approach of companies in North America 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, 8:15-9:45. P4

DIS. OF BENEFICIAL INVERTEBRATES 2

Moderator: Lena Poppinga

8:15  128 Nosima ceranae News: Update on Species Competition and Host-Pathogen Interaction Studies Leelien Solter, Zachary Huang, Wei-Fone Huang and Meghan Milbraith, Illinois Natural History Survey, University of Illinois; Michigan State University

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

8:45  130-STU The involvement of bumblebee small interfering RNA pathway against two different bee viruses Jinzi Li, 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 Ermewein, 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, Richard Gallinier, Guillaume Motta, Benjamin Gourbal, CNRS, UMR 5244, Ecologie et Evolution des Interactions (2EI), Perpignan, France; Université de Perpignan, Perpignan, France

9:30  133-STU A first report of an immune-associated cytosolic PLA2 in insects: Gene structure and function Jiyeong Park and Yonggyun Kim; Department of Bioresources Sciences, Andong National University, Andong, Korea
### FUNGI 4

**Moderator:** Richard Humber and Annette Brunn Jensen

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<tr>
<th>Time</th>
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<tr>
<td>8:00</td>
<td><strong>134</strong> Fungal dimorphism in the entomopathogenic fungus <em>Nomuraea rileyi</em>: A search for <strong>in vivo</strong> produced quorum-sensing molecules</td>
<td>Boucia, Drion, Liu, Shouzou, and Baniszewski, Julie; Entomology and Nematology Department, University of Florida, Gainesville FL, USA; Agricultural College, Liaocheng University, Liaocheng, Shandong, China</td>
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<td>8:15</td>
<td><strong>135</strong> Multilocus genotyping of <em>Amylostereum</em> spp. associated with <em>Sirex noctilio</em> and other woodwasps from Europe reveal clonal lineage introduced to the US</td>
<td>Louela A. Castrillo, Ann E. Haak, Ryan M. Kepler, Juan A. Pajares, Iben M. Thomsen, Gyorgy Csodka, Paula Zamora, and Sergio P. Angelii; Department of Entomology, Cornell University, Ithaca, USA; Sustainable Forest Management Research Institute, University of Valladolid, Palencia, Spain; Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark; Department of Forest Protection, Forest Research Institute, Mátáfrüd, Hungary; Calabazanos Forest Health Center, Castille and Leon, Palencia, Spain; Faculty of Science and Technology, University of Bolzano, Italy</td>
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<td>8:30</td>
<td><strong>136</strong> Preliminary analysis of the genome sequence of <em>Beauveria caudalonica</em></td>
<td>Travis R. Glare, Aimee C. McKinnon, and Murray P. Cox; Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand; Massey University, Palmerston North, New Zealand</td>
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<td>8:45</td>
<td><strong>137</strong> MALDI-TOF Mass Spectrometry: A complement to sequence-based identification technologies for major fungal entomopathogens</td>
<td>Richard A. Humber, Rogério Biaggioni Lopes, Marcos Faria; USDA-ARS Biological IPM Research, RW Holley Center, Ithaca, New York, USA; Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil</td>
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<td>9:00</td>
<td><strong>138</strong> Transcriptomic study reveals <em>Pandora formicae</em> expressing pathogenicity related genes in final stages of host infection</td>
<td>Joanna Malagocka; Morten N. Grell, Lene Lange, Jørgen Eilenberg, Annette Bruun Jensen; Centre for Social Evolution, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Copenhagen, Denmark</td>
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<td>9:15</td>
<td><strong>139</strong> Transcriptome analysis of the entomopathogenic oomycete <em>Lagenidium giganteum</em> reveals putative virulence factors shared by fungal and oomycete entomopathogens</td>
<td>Paul F. Quiroz Velasquez, Sumayyah Abiff, Quincy B. Conway, Norma C. Salazar, Ana Paula Delgado, Jhanelle K. Dawes, Lauren G. Doura, Aurélien Tartar; Nova Southeastern University, Fort Lauderdale, FL, USA</td>
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**Symposium 6** (Bacteria)

**Organizers/Moderators:** Ken Narva and Colin Berry

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<th>Time</th>
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<td>10:30</td>
<td><strong>140</strong> Structural and biophysical characterization of <strong>Cry34Ab1</strong> and <strong>Cry35Ab1</strong>: A search for <strong>novel</strong> insecticidal toxins</td>
<td>Matthew S. Kelker, Colin Berry, Matthew D. Baker, Steven L. Evans, Reetal Pai, David Maggs, Joshua C. Russel, Nick X. Wang, J.W. Pflugrath, Cheng Yang, Matthew Wade, Tim J. Wess, Kenneth E. Narva, Dow AgroSciences, LLC, Indianapolis, Indiana, USA; Cardiff School of Biosciences, Cardiff University, Cardiff, Wales, UK; Rigaku Americas Corporation, The Woodlands, Texas, USA; School of Optometry &amp; 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</td>
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<td>11:10</td>
<td><strong>141</strong> Insights into the structures of non-3-domain toxins through structural modelling</td>
<td>Colin Berry; Cardiff School of Biosciences, Cardiff Univ., Cardiff, UK</td>
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<td>11:30</td>
<td><strong>142</strong> Novel MTX Toxins for Insect Control</td>
<td>Yong Yin; Monsanto Company, St. Louis, MO, USA</td>
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<td>11:50</td>
<td><strong>143</strong> Insecticidal toxins from <em>Photobacterium luminescens</em> and <em>asymbiotica</em>, targeting the actin cytoskeleton and GTP-binding proteins</td>
<td>Thomas Jank, Alexander E. Lang and Klaus Aktories; Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Freiburg, Germany</td>
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<td>12:10</td>
<td><strong>145</strong> Molecular basis of parasporin-2 action toward cancer cells</td>
<td>Sakae Kitada, Yusuke Yoshida, Yoshimi Ozaki, Hironayu Shimada, Kyushu Institute of Technology, Iizuka, Japan</td>
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**MICROBIAL CONTROL 2**

**Moderator:** Surrendra Dara

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<th>Time</th>
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<tr>
<td>10:30</td>
<td><strong>146</strong> Evaluation of the non-target effects of <em>Bacillus thuringiensis</em> subspecies <em>israelensis</em> in standardized aquatic microcosms</td>
<td>Irene Ketseoglou; Gustav Bouwer, School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa</td>
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<td>10:45</td>
<td><strong>147</strong> <em>Bacillus thuringiensis</em> 00-50-5 strain with high activity against plant-parasitic nematodes and insect pests</td>
<td>Cheng Bai, Haibo Long, Leping Liu, Yanling Yang, Jianjun Yue; Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haitou, Hainan, China; North University of China, Taiyuan, China</td>
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<td>11:00</td>
<td><strong>148</strong> Investigations on residues of <em>Bacillus thuringiensis</em> on tomato</td>
<td>Dietrich Stephan; Heike Scholz-Dobin, Hans Kessler, Theo Rentjes, Andreas Truis, Julius Kühn-Institute, Darmstadt, Germany; Landwirtschaftskammer Nordrhein-Westfalen, Germany</td>
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11:15 149 Biological control of western corn rootworm larvae (Diabrotica virgifera virgifera) with Dianem® (Heterorhabditis bacteriophora) Ralf-Udo Ehlers, e-nema, GmbH, Schwentental, Germany

11:30 150 Evaluation of Ten Plant Extracts as Ultraviolet Protectors for Spodoptera littoralis nucleopolyhedrovirus Koko Dwi Sutanto, Saïd El Salamouny, Martin Shapiro, Merle Shepard, Sukirno Miharjo, Muhammad Tufail, Khawaja Ghulam Raezool 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, Saska van Nouhuys, 1Department of Entomology, Cornell University, Ithaca New York, USA; 2Department of Biosciences, University of Helsinki, Helsinki, Finland

12:00 152 Oryctes rhinoceros population diversity and potential implications for control using Oryctes nudivirius Sean D.G. Marshall, Aubrey Moore, Russell K. Campbell, Roland J. Quiluguia, Trevor A. Jackson, 1Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch, New Zealand; 2College of Natural and Applied Science, University of Guam, USA; 3Biosecurity 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 Yazıcı, Gülengül Duman, İsmail Aslan, Burçin Asutay, Tuğçe Palamut, Sıdıka Tapşın, Fikrettin Şahin, 1Department of Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey; 2Faculty 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, B. Cameron Donly, David A. Thielmann, Dwayne D. Hagedus, Cathy Coutu and Douglas Baldwin, 1Saskatoon Research Centre, AAFC, Saskatoon, Canada; 2Southern Crop Protection & Food Research Centre, AAFC, London, Canada; 3Pacific 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 Labrouse; Elisabeth Herniou, Institut de Recherche sur la Biologie de l’Insecte, Université François Rabelais de Tours, Faculty 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, Faculty 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, Marion Ballenghien, Vincent Cahais, Nicolas Galtier, Elisabeth Herniou, Philippe Gavray, 1Institut de Recherches sur la Biologie de l’Insecte, CNRS UMR 7251, Université François-Rabelais, Tours, France; 2Université Montpellier 2, Institut des Sciences de l’Évolution de Montpellier, Montpellier, France

11:30 158 Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons Clément Gilbert, Aurélien Chateigner, Lise Erenwein, Valérie Barbe, Annie Bézier, Elisabeth A. Herniou, and Richard Cordaux, 1Université de Poitiers, Ecologie et Biologie des Interactions, Équipe Écologie Évolution Symbiose, Poitiers Cedex, France; 2Université François-Rabelais de Tours, Tours, France; 3Laboratoire 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, Daniel L. Rowley, Melody Keena, 1Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, USDA Agricultural Research Service, Beltsville, Maryland, USA; 2Northern Research Station, USDA Forest Service, Hamden, CT, USA

12:00 160 Phylogenomics reveals ecological factors that lead to speciation in Baculoviridae Julien Thézé, Carlos Lopez Vaaonide, Jennifer S. Cory, Elisabeth A. Herniou, 1Université François-Rabelais, UFR Sciences, Tours, France; 2INRA, Zoologie Forestière, Orléans, France; 3Dept 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, Shengyong Wu, Zhongren Lei, Xuenong Xu, 1State 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-Más, Lola Ortega-García, Carlos Campos-Porcuna, Inmaculada Garito-Jurado, Enrique Quesada-Moraq; University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain
11:15 165-STU Recovery and detection of an entomopathogenic endophyte: overcoming the challenges involved Aimee McKinnon, Travis Glare, Hayley Ridgway, Andrew Holoayaki, 1Bio-Protection Research Centre, Lincoln University, Christchurch, New Zealand; 2Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand

11:30 166-STU Intense spatial-temporal pattern in pathogen-host interaction between Pandora formicariae and Formica rufa Joanne Malagocka, Jorgen 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, Jorgen Eilenberg, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

12:00 168-STU Plant volatile organic compound manipulation by entomopathogenic fungi Aragon, Sandra, Cotes, Alba Marina, Vidal, Stefan, 'Georg-August-Universitét Göttingen, Department of Crop Sciences, Göttingen, Germany; 1Biotechnologie and Bioindustry Center, Colombian Corporation for Agricultural Research Corpoica, Mosquera, Colombia

12:30–14:00 LUNCH Mensa

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

JIP EDITORIAL BOARD

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

HOW TO WRITE A PAPER
Moderators: Rich Humber, Mark Goettel and Yukino Inoue

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

MICROSPORIDIA 1
Moderator: Susan Bjersom

14:00 169 Effects of the microsporidium Nosema adaliae on the multicoloured Asian lady beetle, Harmonia axyridis Bryan Ellis, Susan Bjersom, 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 Bjersom, 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. Beccel, Christopher Desjardins, Neil Sanzcorriente, and Cristina Cuomo; 1Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL, USA; 2Genome 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, R. Kerr, D. Wriedu-Boakye, B. Williams, G.D. Stentiford; 1European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset, UK; 2Biosciences, University of Exeter, Devon, UK

15:00 173-STU Understanding the evolutionary loss of glycolysis in intranuclear crab microsporidians Dominik Wriedu Boakye, Bryony Williams, Grant Stentiford, and Thomas Williams, 1College of Life and Environmental Sciences, University of Exeter, Exeter, UK, 2Centre of Environment, Fisheries and Aquaculture Science, CEFAS, Weymouth, UK; 3Institute 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, Sebastian Gisder, Elke Genersch, Andreas Linde, 1Hochschule für nachhaltige Entwicklung Eberswalde, Dept. of Forest and Environment, Eberswalde, Germany, 2Länderinstitut für Bienenkunde Hohen Neudorf e.V., Hohen Neudorf, Germany, 3Freie 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, Vladimir Voronin, Egor Rusakovich, Irena Issi, 1All-Russian Institute of Plant Protection, St. Petersburg, Russia; 2St. Petersburg Veterinary Medical Academy, St. Petersburg, Russia; 3Herzen State Pedagogical University of Russia, St. Petersburg, Russia

14:15 178-STU Synthesis and Characterization of fungus mediated silver nanoparticles for the toxicity on filarial Vector, Culex quinquefasciatus Siwa Kamalakannan, Chandrakasan Gobinath, Sivapurnam Ananth, Kadarkarai Murugan, 1Division of Entomology, Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India; 2Bio control laboratory, Department of Biotechnology and Genetic Engineering, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India; 3Insect 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 Mufthah Akhavat; Katharina Döl, 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 sial weevil: The less the better? Vasiliik Gkouni, Markogiannaki Dimitra, Dimitris Kontodimas, 1SLU, Sweden, 2Benaki Phytopathological Institute, Greece
15:10 181 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. Lobo1,2, Everton K. K. Fernandes1, Christian Luz2, M. Patricia Juárez1, Nicolas Pedrini1, 1Instituto de Investigaciones Bioquimicas de La Plata (CCT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, La Plata, Argentina; 2Instituto de Patología 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-Moraga1, Inmaculada Gariño-Jurado2, Meeiad Youssef3, 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 Barbercheck1, Christina Mullien1, Department of Entomology, Penn State University, University Park, USA

Contributed Papers Wednesday, 14:00-16:00  P5

VIRUSES 5

Moderators: Bryony Bronning and Alicia Timm

14:00 184 Soybean aphid viruses exploit contrasting transmission strategies Diveena Vijayanand1, Siju Liu1, Bryony C. Bronning1, Department of Entomology, Iowa State University, Ames, USA

14:15 185 Characterization of mechanisms involved in the transmission of a lepidopteran densovirus Cécilia Mulleau1, Doriane Mutuel2, Manuela Rakotomanga3, Anne Kenaghat4, Clément Bousquet5, Rémy Froissart6, Nathalie Volkoff7, 1INRA, UMR 1333 DGIMI, INRA, Montpellier, France; 2CNRS, UMR 5290 MIVEGEC, Montpellier, France; 3CNRS, UMR 5290 MIVEGEC, Montpellier, France

14:30 186 Discovery of circular single-stranded DNA viruses in top insect predators Karyna Rosario1, Anisha Dayaram1, Jessica Ware2, Milen Marinov2, Mya Breitbart1, Arvind Varsani3, 1College of Marine Science, University of South Florida, Florida, USA; 2School of Biological Sciences, University of Canterbury, Christchurch, New Zealand; 3School of Environmental and Biological Sciences, Rutgers University, New Jersey, USA

14:45 187-STU Single-stranded DNA viruses in marine crustaceans Ryan Schenck1, Karyna Rosario1, Rachel Harbeltner1, John Cannon1, Mya Breitbart1, 1University of South Florida College of Marine Science, Tampa, Florida, USA; 2University 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 Yu1, Max Bergoin2, and Peter Tjissen3, 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. Pham1, Qian Yu1, Max Bergoin2, Peter Tjissen3, 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 Timm1 and Annette Reineke2, 1Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa; 2Institut für Phytomedizin, Geisenheim Hochschule, Geisenheim, Germany

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

BACTERIA 4

14:00 192 Analysis of the bacterial community of the insect pest Lymantria dispar during its life cycle Zane Metta1,2, Monika Maurhofer3, Liga Jankewica1,2, 1Plant Pathology, Institute of Integrative Biology (IBZ), Swiss Federal Institute of Technology, Switzerland; 2Laboratory of Experimental Entomology, Institute of Biology, Univ. Latvia, Latvia; 3Univ.of Daugavpils, Latvia

14:15 193 Contacting microbe induce grooming behaviour in Drosophila Aya Yanagawa1,2, Tsuyoshi Yoshimura1, Hata Toshimitsu1, Frédéric Marion-Poll3, 1Kyoto University, Uji, Japan; 2CNRS, Laboratoire Evolution, Génomes et Spéciation, Gil-sur-Yvette, France; 3AgroParisTech, Département Sciences de la Vie et Santé, Paros, France

14:30 194 Cultivable gut bacteria of scarabs inhibit B. thuringiensis multiplication Yueming Shan1,2, Changlong Shu1, Neil Crickmore3, Chunqiu Liu2, Wensheng Xiang1, Fuping Song1, Jie Zhang1, 1School of Life Science, Northeast Agricultural University, Harbin, P.R. China; 2State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China; 3School of Life Sciences, University of Sussex, Falmer, Brighton, UK; 4Cangzhou Academy of Agricultural and Forestry Sciences, Cangzhou, P.R. China

14:55 195 Interactions between the Med fly Ceratitis capitata (Wied.) and a new Bacillus cereus sensu lato strain Luca Ruiu1,2, Giovanni Falchi1, Ignazio Floris1, Maria G. Marche1,2, Maria E. Mura1, Alberto Satta1, 1Dipartimento di Agraria, University of Sassari, Italy; 2Bioecopest 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 Schneider1, Tania Tajín1, Niels B. Hendriksen2, Jan O. Lundström1, Petter Melin1, Ingvar Sundh1, 1Department of Microbiology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; 2Department of Environmental Science, Aarhus University, Roskilde, Denmark; 3Mosquito 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. Marche1,2, Maria E. Mura1, Giovanni Falchi1, Luca Rulı1,2,1 Dipartimento di Agraria, University of Sassari, Italy, 2Biocopeast 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. Vanhove1, Marylise Dupertuy1,2, Guillaume M. Charnière1, Frédérique Le Roux2, David Goudenège1,2, Benjamin Gourbal1, Sylvie Kieffer-Jaquinod1, Yohann Couté1, Sun N. Wai3 and Delphine Destoumieux-Garzon4,1 Ecology of coastal marine systems, CNRS, Ifremer, France; 2IRMAR, University of Montpellier, France; 3University of Nantes, Faculty of Biology and Chemistry, France; 4UMR Eco and Evolution of Interactions, France; 5Université Grenoble-Alpes, CEA, IRTSV, Biologie à Grande Échelle; 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 Nalcacioğlu, Ismail Demir, Hüseyin Tepe, İsmail Yıldız, Ardahan Eski, Zihni Demirbağ, 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. Kleespies1, Andreas Leclerque2,1 Institute for Biological Control, Julius Kühn Institute (JKI), Germany, 2Geisenheim 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 Fürnhaus, Elke Genersch Institute 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, Flavia Büchler, Maria Péchy-Tarr, Peter Kupferschmied, Monika Maurohofer, and Christoph Keel, 1Department of Fundamental Microbiology, University of Lausanne, Switzerland, 2Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland

**BA-5-STU** Identification and Characterization of *Bacillus thuringiensis* Strains with Nematicidal Activity
Luis A. Verduzo-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. Tripathi1, A. Kumar2, L. Saravanan3 G.T. Guj4, 1Division of Entomology, Indian Agricultural Research Institute, New Delhi, 2TERI, India Habitat Centre, New Delhi, 3Directorate of Medicinal and Aromatic Plants Research, Anand

**BA-7** Gene organization of large plasmids of novel mosquicidal *Bacillus thuringiensis* TK-ES
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 Ilıgo Ruiz de Escudero1,2, Núria Banyuls3,1, Yolanda Bel3,1, Mireya Maetzu1, Baltasar Escriche1,3, Delia Muñoz1,2, Primitivo Caballero1,2, Juan Ferré3,1, Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadia, Mutxila, Navarra, Spain, 2Laboratorio de Entomología Agrícola y Patología de Insectos, Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain, 3Departamento 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 Lemes1, Camila Chiaradavolos1, Paula Cristina Brunini Crialesi Legori2, Odair Aparecido Fernandes2, Juan Ferré3, Manoel Victor Franco Lemos,1 Janeté Apparecida Desiderio1,1 Dpto de Biología Aplicada a Agropecuaria, UNESP/Campus de Jaboticabal, Brazil, 2Dpto de Fitossanidade, UNESP/Campus de Jaboticabal, Brazil, 3Dpto de Genética, Universidade de Valência, Valencia, Spain

**BA-10** Cry1Ac and Cry1F toxicity and binding sites study in two important soybean pests, *Anticarsia gemmatalis* and *Chysodeixis (=Pseudoplusia) includens*.
Yolanda Bel1, Ken Narva2, Joel Sheet2, Baltasar Escriche1,3, 1Dept. Genetics, ERI BioTecMed, Universitat de València, Dr. Moliner, Burjassot, Valencia, SPAIN, 2Dpt. 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 125I-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 Abdelgafar1,2, Cris Oppert1,2, Jayme Williams1,2, Deepa Balasubramanian1,2, Juan Luis Jurat-Fuentes1,2, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville (TN), USA, 1Bayer 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 Aminomentinidase N 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 Youngpin Park and Yanggyun 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 Youngpin 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 Chen1,2, Changlou Shu1, Jacob Evans2, Fuping Song1, Guoxun Li1, Neil Chckmore3, Jie Zhang1, State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P. R. China; 2School of Life Sciences, University of Sussex, Falmer, Brighton, UK; 3College of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao, China

BA-18 Bacillus thuringiensis Cry3Aa toxin increases the susceptibility of Cricotris quatuordecimpunctata to Beauveria bassiana infection Yulin Gao1, Zhongren Le1, Xunong Xu1, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, P.R. China

BA-19 InterVening Sequence (IVS) elements as genetic markers for the differential diagnosis of arthropod-associated Rickettsiella bacteria Christina Schuster1; Katharina Schär1; Regina G. Kiesbies1; Andreas Leclerque2; 1Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; 2Institute 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 Piech-Tarr1, Nicolas Wenner2, Peter Kupferschmid1, Romane Keller1, Monika Mauroher2, Christoph Keel1. 1Department of Fundamental Microbiology, University of Lausanne, Switzerland; 2Plant 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 Kniepsel, Lena Poppinga, Anne Fürnhaus, Elke Genersch, Institute for Bee Research, Hohen Neudorf; Division of Molecular Microbiology and Bee Pathology, Hohen Neudorf, 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 Patrício Navarrete1,2, Isabelle Jéhanne1,2, Christina Nielsen-Leroux1,2 and Vincent Sanchis1,2. 1INRA, UMR1319 Micalis, F-78350 Jouy-en-Josas, France; 2AgroParisTech, 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 Brite Art Gillian Hertlein1, Lena Poppinga2, Eva Garcia-Gonzalez2, Elke Genersch1,2, 1Institute for Bee Research Hohen Neudorf, Hohen Neudorf, Germany; 2Technische Universität Berlin, Institute of Biotechnology, Berlin, Germany; 3Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany

DB-2 Awareness and Concept of Insects in a Korean Population Sungh Min Bang, 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. Burand1, Matthew Boucher2, Anne Averill2, Departments of Microbiology, Biology and Environmental Conservation, University of Massachusetts - Amherst, Amherst, USA

DB-4 Honeybee Virus Epizootiology in Bee Populations in Connecticut, USA John P. Burand1, Shuning Zheng1, Kimberly Stoner2, 1Department of Microbiology, 2Graduate Program in Molecular and Cellular Biology, University of Massachusetts - Amherst, Amherst, USA and 3Connecticut 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 Hutton1, John A. Hammond2, School of Ocean and Earth Sciences, University of Southampton, National Oceanography Centre, Southampton, Hants, UK; 2Immunogenetics Group, The Pirbright Institute, Pirbright, Woking, UK

DB-6 A novel pathogenic Paenibacillus strain of Biomphalaria glabrata, an intermediate host for schistosomiasis David Duval1,2, Richard Galinier1,2, Gabriel Mouahid1,2, Eve Toulza1,2, Anne Rognon1,2, Nathalie Arancibia1,2, Jean Francois Allienne1,2, Guillaume Milla1,2, André Théron1,2, Benjamin Gourbal1,2, CNRS, UMR 5244, Ecologie et Evolution des Interactions (2EI), Perpignan, France; 1Université 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 larve Natalia A., Kryukova1, Ekaterina A., Chertkov1, Alexandra D., Semenova1, Yuri I., Glazachev2, Irina A., Slepneva2, Victor V., Glupov1, 1Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia; 2Institute 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 Poppinga1, Heike Aupperle2, Elke Genersch1, 1Institute for Bee Research, Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany; 2Laboklin GmbH & Co KG, Bad Kissingen, Germany

DB-9 New findings in genome of Apis mellifera filamentous virus Lukáš Rabalski, Urzula Grzeda, Grażyna Topolska, Martyna Krejmer, Boguslaw Szewczyk, 1Department of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology of the University of Gdansk and Medical University of Gdansk, Gdansk, Poland; 2Laboratory 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 Ragni1, Francesca Tabarrini1, Mario Carucci1, Claudio E. Lorenzetti1, Antonella Cersini2, Silvia Puocica2, Valeria Antognetti2, Marcella Millito2, Alessandra Giacomelli2, Giovanni Formato2, Francesco Panara1, 1RAPID BIOTECH, Perugia; 2Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma; 1ENEA - 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? Stefentford, 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

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

FU-2 Distribution of insect-pathogenic soil fungi in agricultural and forest ecosystems in Georgia Medea Burjanadze, Richard Humber, Mariam Arjavanidze, Tea Abramishvili, Giuli Tsereteli, Manana Lortkipanidze, 1Agricultural University of Georgia, Department of Forest protection, Georgia; 2USDA-ARS BioPM Resarch, RW Holley Center for Agriculture and Health, Ithaca, NY., USA; 3IIs 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 Delalibéra Jr. Department of Entomology and Acsarology, 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 Saar1, Andreas Leclerque2, Dietrich Stephan, 1Institute for Biological Control, Julius Kühn-Institut (JKI), Darmstadt, Germany; 2Institute 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, Polina V. Mitkovets, Galina V. Mitina, Alexandru Movila, Yuri S. Tokarev, 1Institute of Zoology, Academy of Sciences of Moldova, Chisinau, Republic of Moldova; 2All-Russian Institute for Plant Protection, Saint-Petersburg, Russia; 3Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; 4Institute 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, Livia Maria Alves Porto, Jérgen Eilenberg, Italo Delalibera Junior, 1University of São Paulo (ESALQ), Brazil; 2Department 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, Stefan Stranne, Hanna Frīberg, Ingvar Sundh, 1Department of Microbiology and 2Department 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 Katerina Šimáčková, Jana Kročáková, Noemi Herrera, 1Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i. Institute of Entomology, České Budějovice, Czech Republic; 2University of South Bohemia, Faculty of Agriculture, České Budějovice, Czech Republic

FU-9 Entomopathogenic fungi as plant growth enhancers Surendra K. Dar1, Sumanth S. Dar1, Suchitra S. Dar1, 1Division of Agriculture and Natural Resources, University of California; 2Stockdale High School, Bakersfield, USA; 3Warren Junior High School, Bakersfield, USA.

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

FU-11-STU Interactions between cowpea plants vs. Metarhizium spp. entomopathogenic fungi Patricia S. Golo, Walquiria Arruda, Flávia R. S. Paixão, 1Universidade Federal de Viçosa, Brazil; 2Universidade Federal de Viçosa, Brazil.
FU-12 Biological control in oilseed rape: An attempt to establish the entomopathogenic fungus Beauveria bassiana as an endophyte in oilseed rape plants
Comellia Ulrich1; Saussene Tabli2; Andreas Leclerque2; Frank Rabenstein2; Regina G. Klessiespies1; 1Institute for Biological Control, Julius Kühn Institute (JKI), Germany; 2ESALQ – University of São Paulo, Piracicaba, Brazil; 3USDA-ARS BiopIM Research, Ithaca, NY, USA.

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 Westrum1; Vanessa S. Duarte1; Richard A. Humber1; Ivalo Delalibera Jr.1; Ingeborg Klingan1; 1Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Ås, Norway; 2ESALQ – University of São Paulo, Piracicaba, Brazil; 3USDA-ARS BiopIM Research, Ithaca, NY, USA.

FU-14 Susceptibility of Biomphalaria glabrata egg masses to fungal infection
Glennyya F. Duarte, Juscelino Rodrigues, Everton K. K. Fernandes, Christian Luz; 1Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil; 2USDA-ARS BiopIM Research, Ithaca, NY, USA.

FU-15 Antimicrobial, Antioxidant and Anticancer Activity of Culture filtrates from Entomopathogenic Fungi Tae Young Shin, Sun 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 robertsi 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 robertsi ARSEF 2575 Flávia R. S. Paixão1; Elen R. Muniz2; Cintia C. Bernardo1; Gabriel M. Mascarin2; Christian Luz1; Everton K. K. Fernandes1; 1Universidade Federal de Goiás, Goiânia, Brazil; 2Embrapa Arroz e Feijão, Goiânia, Brazil.

FU-18 Delayed germination of heat-stressed conidia of Metarhizium anisopliae on tick cuticle Lucas P. Barreto1; Fabricio M. Alves1; Christian Luz2; Gabriel M. Mascarin2; Donald Roberts2; Walquiria Arruda3; Everton K.K. Fernandes1; 1Universidade Federal de Goiás, Goiânia, Brazil; 2Embrapa Arroz e Feijão, Goiânia, Brazil; 3Utah 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. Araujo1; Clara C. Oliveira1; Marilia A. Rodrigues1; Breno Pupin1; Luciana P. Dias1; John E. Hallsworth1; and Drauzio E. N. Range1; 1Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraiba, São José dos Campos, Brazil; 2School 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 robertsi Luciana P. Dias1, Drauzio E. N. Range1; 1Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraiba, 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 Parth1, Judith Taibon1, Hermann Strasser1; 1Institute of Microbiology, Leopold-Franzens University Innsbruck, Austria; 2Institute of Pharmacy / Pharmacognosy, Leopold-Franzens University Innsbruck, Austria

FU-23 Evaluation of destruxin A production in four strains of Metarhizium by capillary electrophoresis Alex Rios-Moreno1, Azahara Carpio1, Inmaculada Garrido-Jurado1, Lourdes Arce1, Miguel Vaírcel1, Enrique Quesada-Mora2, 1Departament of Environmental and Forestry Sciences, ETSIAM, University of Cordoba, Campus de Rabanales. Edificio C4 Celestino Mutis, Cordoba, Spain; 2Department of Analytical Chemistry, University of Cordoba, Annex C3 Building, Nanochemistry and Fine Chemistry Research Institute (IUIQFN), Campus de Rabanales, Cordoba, Spain

FU-24 Entomopathogenic fungal genera and the 1F=1N standard: The shape of the future begins to emerge Ryan M. Keplar1, Stephen A. Rehner1, Richard A. Humble2, 1USDA-ARS Systematic Mycology and Microbiology Laboratory, Beltsville, Maryland, USA; 2USDA-ARS Biological IPM Research, RW Holley Center, Ithaca, New York, USA

FU-25 Genotyping of Georgian isolates of entomopathogenic fungi Beauveria spp. Nana Kunelaun1; Vladimir Baramidze1; Medea Burjanadze1, Ekaterine Shubladze1, Eka Mikeladze1, 1Agricultural University of Georgia, Tbilisi, Georgia; 2G. Tezvadze Laboratory of Microbial Genomics, Agricultural University of Georgia, Department of Forest Protection, Tbilisi, Georgia

FU-26 Genetic characterization, fungicide sensitivity, and aphidical potential of Lecanicillium fungi from Argentina Romina Manfrino1,2, Christina Schuster3, Julieta Tornesello Galván1, Katharina Saar1, Juan J. García1; Claudia C. López Lastra1; Andrea Leclerque3,4; 1Centro de Estudios Parasitológicos y de Vectores (CEPAVE), La Plata (BsAs), Argentina; 2Instituto Nacional de Tecnología Agropecuaria (INTA), Rafaela (Santa Fe), Argentina; 3Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; 4Institute 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. robertsi Johanna Mayerhofer1; Andy Lutz2, Franco Widmer1; Stephen A. Rehner3, Ryan M. Kepler2, Adrian Leuchtmann2; Jürg Enkerli1; 1Molecular Ecology, Institute for Sustainability Sciences, Agroscope, Reckenholzstrasse Zurich, Switzerland; 2Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland, USA; 3Plant Ecological Genetics, Institute of Integrative Biology, ETH Zurich, Switzerland

M. Alves2; Éverton K. K. Fernandes2; Donald W. Roberts2; Vânia R. E. P. Bittencourt1; 1Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil; 2Universidade Federal de Goiás, Goiânia, Brazil; 3Utah State University, Logan, USA.
FU-28 Species identification of entomopathogenic fungi of the genus *Lecanicillium* (= *Verticillium lecaniellum* 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, John Vandenberg, Michael Griggs, Stephen Wright, Yonathan Estrella, Susan Rottschaefer, Andrew Clark, Brian Lazaro, 1Department of Entomology, Cornell University, Ithaca NY, USA; 2USDA Agricultural Research Service, Ithaca NY, USA; 3Department 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, 1The United Graduate School of Agricultural Sciences, Iwate University, Japan; 2Department of Agro-environmental Science, Ohibrio 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 Kabuluk 1, Manon Peyre 2, Remi Thomasset 2, 1Biologie Department, University of the Fraser Valley, Abbotsford, BC, Canada; 2Agriculture 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, Mzago 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, Vitalis Wafia Wakesa 2, Ingemborg Kängen 2, Italo Delalbera Júri 2, University of São Paulo (ESALQ), Brazil; 2The Kenya Polytechnic University, Kenya; 3Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway, 4The 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 Patil 1; Hermann Strasser 1; Tariq Butt 1; Joergen Ellenberg 1; Juerg Enkerl 1; Enrique Quesada 1; Justus Wesselet 1; Francesca Tencalai 1; Arne Peters 1; Miloslav Nesrta 1; Andrew Shearer 1; Hermann Limbers 2; Erik Hansen 2; Athanasios Koukoutsakis 1; 1Georg-August-Universität Göttingen, Germany; 2University of Applied Sciences, Germany; 3University of Innsbruck, Austria; 4Swansea University, United Kingdom; 5University of Copenhagen, Denmark; 6Agroscope Reckenholz-Tänikon, Switzerland; 7University of Córdoba, Spain; 8Technische Universität München, Germany; 9Troms, Norway; 10Neem Biotech Ltd, United Kingdom; 11Klaasmann-Deilmann GmbH, Germany; 12Fytovita, Czech Republic; 13Neem Biotech Ltd, United Kingdom; 14Klaasmann-Deilmann GmbH, Germany; 15EWW BioProduction Aps, Denmark; 16Torux 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 Bayisisa; Sunday Ekei; Godwin P. Kasaya; Samira Mohamed; John M. Wagacha; and Nguy K. Maniania
1International Centre of Insect Physiology and Ecology (icpe), Nairobi, Kenya; 2School 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 Abreu & 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; Frank Delvigne; Frédéric Francis; 1Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Belgium; 2Bio-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*
Etan Ben-Dov; Galina Gindin; Zvi Mendel; Ariel Zaritsky; Ariel Kushman; 1Department of Life Sciences, Achva Academic College, Israel; 2Department of Entomology, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel; 3Faculty of Natural Sciences, Ben-Gurion University of the Negev, Be’er-Sheva, Israel; 4Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Be’er-Sheva, Israel

MC-5 Selection of entomopathogenic fungi for the control of *Aegrophorus nodipennis* (Coleoptera: Curculionidae) under laboratory conditions
Ernesto Cisternas; Andrés France and Inna Urutia; Instituto de Investigaciones Agropecuarias (INIA), La Cruz, Chile. 1INIA Quilamapu. Chillán, Chile

MC-6 Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* strain HD1
Caroline Placidi De Bortoli; Ricardo Antonio Polanczyk; Neil Crickmore; Rafael Ferreira dos Santos; Alessandra Marieli Vacari and Sergio Antonio De Bortoli; 1Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil; 2Department 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; Caroline Placidi De Bortoli; Ricardo Antonio Polanczyk; Neil Crickmore; Rafael Ferreira dos Santos and Alessandra Marieli Vacari; 1Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil; 2Department of Biochemistry, University of Sussex, Brighton, UK
MC-8 Effect of Bacillus thuringiensis Berliner on biological characteristics of Oryis 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 Falaggiarda, 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 Asial pest, Drosophila suzukii Maria Fernández-Braza, 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, Allan F. Marciano, Filipe A. Sá, Wendell M, S Perinotto, Simone Quinelato, Patricia S. Golo, Isabelle C. Angelo, Mária C. A. Prata, Vânia R. E. P. Bittencourt, Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil; Embrapa Gado de Leite, Juiz de Fora, MG, Brazil

MC-12 Two Colombian entomopathogenic fungi are highly efficient on Cerotoma lingamarina Erika Grijalba, Adriana Santos; Carlos Espinell, Center of Biotechnology and Bioindustry CBB; Colombian Corporation for Agriculture Research, CORPOICA, Quetza, Colombia

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


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, Ameneert 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, Sergio J. Mijalosky, Patricia Juárez, Christian Luz, Everton K. K. Fernandes and Nicolás Pedrini, Instituto de Investigaciones Bioquímicas de La Plata (ICT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, La Plata, Argentina; 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. Melta; Rita Seskena, Santa Volkpane, Monika Maurhofer Briggolf, Liga Jankevica, Laboratory of Experimental Entomology, Institute of Biology, University of Latvia, Latvia; 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 Polanzyk, Caroline Placidi De Bortoli, Neil Crickmore, Rafael Ferreira dos Santos, Alessandra Marieli Vacari and Sergio Antonio De Bortoli, Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil; 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 Polanzyk, Thiago Trevisoli Agostini, Luis Fernanda Moreira, Rogério Teixeira Duarte, Fernando Hercos Valicente, Microbial Control of Pests Lab, Plant Protection Department, Universidade Estadual Paulista, Jaboticabal, Brazil; 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 Yordanyes Ramos González, Ingeborg Klingen, Jorge R. Gómez Sousa, Universidad Central “Marta Abreu de Las Villas (UCLV), Faculty of Agricultural and Animal Science, Villa Clara, Cuba; 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, Hermann Strasser, Roland Zeiger, Institute of Microbiology, Leopold-Franzens University Innsbruck, Innsbruck, Austria; 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, Khonobborn Tangtrakulwanich, Shaohui Wu and John H. Miller, Virginia L. Ophus, Stefan T. Jaronski, Western Triangle Agricultural Research Center, Montana State University, Conrad, USA; 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* (Boisdual) (Lepidoptera: Noctuidae) Gloria Resquin-Romero, Immaculada 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 Roque*; 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 Bernhard M. Steinwender, Jürgen Eilenberg, Elina Panahi, Kiri M. Fleistrup, Marta M. Cáceres, Gabriela M. Vergara, Lena Sigsgaard; Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark

**MC-27** Ultrastructure of midgut of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) strain HD1 Alessandra Marieli Vacari, Vanessa Fabiola Pereira de Carvalho, Caroline Placidi De Bortoli, Ricardo Antonio Polanczky and Sergio Antonio De Bortoli. Department of Plant Protection, Sao Paulo State University, Jaboriocabal, Sao Paulo, Brazil

**MC-28** Control of sugarcane borer, *Diatraea saccharalis*, with formulations of *Beauveria bassiana* and *Metarhizium anisopliae* Inajá M. Wenzel1,2, Antonio Batista Filho3, Moacir R. Forim4, Isabella B. Giordano1, Bárbara E. Denadae1, 1Federal University of São Carlos/ Chemistry Department/ Natural Products Laboratory/São Carlos city, São Paulo state, Brazil; 2Biological Institute/Biological 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* (Microsporidia: 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, Shahajahan 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 Trombík, 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 Malvish, 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 Pilarova1, Danail Takov1, Miroslav Hylis2, Renate Radek3, Leellen Solter4, Andreas Linde5, Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria; 1Faculty of Science, Charles University, Prague, Czech Republic; 2Free University of Berlin, Berlin, Germany; 3Illinois Natural History Survey, University of Illinois, USA; 4University 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 Radek1, Madlen Kantlon1, Jacek Dabert1, Gerd Alberti4, Free University of Berlin, Berlin, Germany; 2Adam Mickiewicz University, Poznan, Poland; 3Ernst-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 Mutis1, Maria F. Achinelli1, Maria V. Micieli2, 1Fellowship CONICET Centro de Estudios Parasitológicos y de Vectores, CEPAVE, La Plata, Argentina; 2Researcher 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. Bornestein-Forst1 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 Cimen; Selçuk Hazir, 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 Hyrls, 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 France1, Ernesto Cisternas2, Irina Urbátil1, 1Instituto de Investigaciones Agropecuarias (INIA), Quilamapu, Chillán, Chile; 2INIA La Cruz, La Cruz, Chile

**NE-7** Nematodes of large larch bark beetle *Ips cembrae*
(Coleoptera: Scolytinae) Sarka Grucmanová¹; Václav Čermák¹, Jaroslav Holuša¹, ², Czech University of Life Sciences Prague; Czech Republic, ³Central Institute for Supervising and Testing in Agriculture, Olomouc, Czech Republic

NE-8 Natural Occurrence of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) in the Aydin district of Turkey
Baris Gulü¹, Canan Hazız¹, Mehmet Karagoz², ³, M. Alper Kesici³, ³, Düzce University, Faculty of Arts and Science, Department of Biology, Düzce, Turkey; ²Aydın Vocational School of Health Services, Adnan Menderes University, Aydın, Turkey; ³Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, Aydın, Turkey

NE-9 Detection of dsRNA virus-like molecules in entomopathogenic nematodes Noemi Herrero; Jiří Nermut; Vladimir 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 Cellular and humoral interactions between the white grub, Polyphylla adspersa Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes Jamileh Avandi¹, ³, Javad Karimi¹, Mohammad Ghadamyari² & Ahmad Asoode², ³, ¹Biocontrol and Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran; ²Department of Plant Protection, College of Agriculture Science, University of Guilan, Rasht, Iran, ³Department 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 Małecki, Joanna Horna, Maria Gawel, Paulina Kramarz, ¹, Institute of Environmental Sciences, Jagiellonian University, 30-387 Krakow, Poland, ²Institute 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 Lee¹; Ho Yul Choo², ¹Major of Applied Biology, School of Ecological Environment and Tourism, Kyungpook National University, Sangju, Republik of Korea; ²Department of Applied Biology, College & Institute of Agriculture & Life Sciences, Gyeongsang national University, Jinju, Republik of Korea

NE-14 Steinernema feltiae (Nematoda: Steinernematidae) to control fungus gnat, Bradysia mabiusi (Diptera: Sciaridae): effect of dosage and application time * Patricia Ballone¹; Luis G. Leite²; Fabio S. Schmidt²; Victória R. Campos¹; Roselaine N. S. Bueno¹; ¹Instituto Biológico, CEIB, CP70, Campinas, Brazil, ²Bio 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 Lorikpanidze, Oleg Gorgadze, Madona Kuchava, Nana Grafiashvili, Mzia Kokha, Nino Gabroshvili, Institute of Zoology, Ilia State University, Tbilisi, Georgia

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 Mikais, 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, Frederiksborg, Denmark

NE-19 Some observation on morphology and ecology of mollusc-parasitic nematode Alloionema appendiculatum Jiří Nermut, Vladimír Půža, Zdeněk Mráček; Biology Centre ASCR v.v.i., Institute of Entomology, Braníčkovská 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, Teodulfo Aquino-Bolaños, ¹Juan R. Delgado and Carlos I. Cortés-Martínez, ²COFAA-IPN y ²PIFI-IPN, Laboratory of Biological Control, CIIDIR U. OAXACA, IPN, Santa Cruz Xoxocotlán, 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
VI-1 High-level Expression of Foreign Protein Using the Partial Polyhedrin-fused Baculovirus Expression System Sung Min Bae; Tae Young Shin; Jae Bang Chol; Yeon Ho Je; Byung Rae Jin; Soo Dong Woo; 1Department of Agricultural Biology, Chungbuk National University, Chungju, Korea; 2Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Korea; 3College of Natural Resources and Life Science, Dong-A University, Busan, Korea

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

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

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

VI-5 Proteomic analysis of the occluded Tipula olereca nudivirus (ToNV) Annie Bézier, Grégoire Harichaux; Julien Gaillard; Karine Musset; Valérie Labas, Lionel Galibert, 1Institut de Recherche sur la Biologie de l’Insecte, CNRS UMR 7261, Université François Rabelais, France; 2Laboratoire de Spectrométrie de masse, Plateforme d’Analyse Intégrative des Biomolécules et des Phénotypes des Animaux d’Intérêt Bio-agromique, UMR INRA 0085-CNRS 7247-UFRE-IFCE, Nouzilly, France; 3Laboratoire de Biologie Cellulaire, Microscopie Électronique, 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*; Joseph S. Elkinton; John P. Burand; Lina Tian; Leelien F. Solter; 1Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, USA; 2Department of Environmental Conservation, University of Massachusetts Amherst, USA; 3Department of Microbiology, University of Massachusetts Amherst, USA; 4Department 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 differential gene expression of Trichoplusia ni granulovirus (TnGV) Angeles Bivian Hernández; 1Ingrid Zamella-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 IV-6 expressing the Cn-10 neurotoxin from Centrurides noxius scorpion Flor C. Arellano-Villagómez; Jorge E. Ibarra; M. Cristina Del Rincón-Castro; 1Food Department, Division of Life Sciences, University of Guanajuato, Irapuato, Gto. México, 2CINVESTAV-IPN Unidad Irapuato, Irapuato, Gto. México

VI-10 Genomic sequencing and analysis of Sucra jujuba nucleopolyhedrovirus Xiaoping Liu, Felfei 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 Yesim Aktürk Düzman; 1,2 Cemal Sandalli, Zihni Demirbağ and Remziye Nalçacıoğlu, 1Karadeniz Technical University, Faculty of Sciences, Department of Biology, Trabzon, Turkey; 2Recep Tayyip Erdoğan University, Faculty of Arts and Sciences, Department of Biology, Rize, Turkey

VI-12 Identification of a new multiple nucleopolyhedrovirus isolated from the Jasmine moth, Palpita unionalis (Hübner) (Lepidoptera: Pyralidae) in Egypt Regina G. Kliespies, Youngie Wang, Said El Salamouny, Mona Awad, Essam Agamy, Ramadan Salama and Johannes A. Jehle*; 1Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; 2Agricultural Service Station Palatinate, Neustadt/Weinstr., Germany; 3Department 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 Gilibert; Aurélien Jacob; Bérangère Bertin; Marjorie Boutin Fontaine; Delphine Bonnin; Christophe Lecomte; Christel Rivière; Otto-Wilhelm Merten; Genevthon, 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 Nucleopolyhedrovirus Leo Graves*; Farheen Raza, Sarah L. Irons; 1Robert D Possee* and Linda A King, 1Department of Biological and Medical Sciences, Oxford Brookes University, Oxford UK, 2Oxford 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*; M. Leticia Ferrelli; 1Matias L. Pidre; Alicia Sciocco-Cap; 1Victor Romanowski, 1IBBM-UNLP-CONICET, La Plata, AR; 2IMyZA-INTA, Castellar, AR
VI-16 Enhancing Genes of *Lymatia dispar* NPV Do Not Increase Potency Via Metalloprotease Activity Kelli Hoover1, James Slavicek2, Aligimitas P. Valaitis3,4, Nancy Hayes-Plazolles5, and Elizabeth McCarthy6,  
1Department of Entomology, Penn State University, University Park, PA USA; 2USDA Forest Service, Delaware, OH USA; 3Retired

VI-17 A Cypovirus VPS 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* gene chimera in *Trichoplusia ni* larvae Miguel A. Salas-Marina1, Cristina Del Rincón-Castro2 and Jorge E. Ibarra3,  
1CINVESTAV-Irapuato, Irapuato, GTO, Mexico; 2División de Ciencias de la Vida, Universidad de Guanajuato, Irapuato, GTO, Mexico

VI-19 iLOV baculovirus: Using a novel small fluoresce protein for imaging virus proteins during infection Farheen Raza1, Sarah Iong2, Leo Graves2, Stan Botchway3, Robert Possel4 and Linda King5,  
1Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; 2Central Laser Facility, STFC, Harwell, UK; 3Oxford Expression Technologies, Oxford, UK

VI-20 Expression analysis of the *nsd-2* gene encoding the putative densovirus receptor in the midgut Katsuhiko Ito1, Hiroko Tabunoki1, Takeshi Yokoyama1, Keiko Kadono-Okuda2, Tokyo University of Agriculture and Technology, Tokyo, Japan; 3National Institute of Agrobiological Sciences, Ibaraki, Japan

VI-21 Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua* Aasa K. Jakubowska1, Melania D Angiolo1, Rosa M. González Martínez2, Anabel Millán Leiva2, Arkaitz Carballo2, Rosa Murillo2, Primitivo Caballero2, Salvador Herrero2,  
1Department of Genetics, Universitat de València, Dr Moliner 50, 46100 Burjassot, Spain; 2Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, 31192 Multiva Baja, Navarra, Spain

VI-22-STU A novel baculovirus-derived promoter with high activity in the Baculovirus Expression System María Martínez-Solís1, Silvia Gomez-Sebastián1, Jose M Escribano1, Agata K. Jakubowska2, Salvador Herrero3,  
1Department of Genetics, Universitat de València, Burjassot, Spain; 2Alternative Gene Expression S.L. (ALGENEX), Centro Empresarial, Parque Científico y Tecnológico de la Universidad Politécnica de Madrid, Campus de Montegancedo, Madrid, Spain; 3Departamento 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 Ozen1, Hacer Muratoglu1, Zihni Demirbag1, Just M. Vlak2, Monique M. van Oers3, Remzye Nalcacioglu1, Karadeniz Technical University;  
1Faculty of Science, Department of Biology, Trabzon, Turkey; 2Karadeniz Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Trabzon, Turkey; 3Laboratory 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 *Cryptoplea leucotreta* granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa John K. Opoku-Debrah1; Martin Hill2; Sean Moore3, Caroline Knox4, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa; 2Citrus Research International, Humewood, Port Elizabeth, South Africa; 3Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, South Africa; 4River 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 nucleopolyhedrovirus in various populations of gypsy moth *Lymantia dispar* Olga Polenogova1, Alexandr Iljinikh1, Dmitriy Kureshchikov1, Philipp Iljinik1, Elena Imranova1, Alexandr Baburin1,  
1Institute of Systematics and Ecology of Animals Siberian Branch of Russian Academy of Sciences, Novosibirsk, RUSSIA; 2Institute of Water and Ecological Problems Far Eastern Branch of Russian Academy of Sciences Kim-Yu-Chena, Khabarovsk, RUSSIA; 3State Research Center of Virology and Biotechnology “Vector”, Novosibirsk, RUSSIA

VI-28 Establishment of SeMNVP Persistent Infection and Screening of Persistent Infection Associated Genes in Baculovirus *Weng Qibei1, Li Min2, Yang Kai2, Pang Yi2*; School of Life Sciences, Guizhou Normal University, Guiyang, China; 2State 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 Shiorgi Sagawa, Eiko Arai, Maki Inoue, Yasuhashi 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 Beperet1, Oihane Simón2, Trevor Williams3; Miguel López-Ferber3, Primitivo Caballero4,  
1Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutiliva, Spain; 2Instituto de Ecología AC, Xalapa, Mexico; 3LGEI, Ecole de Mines d’ Alès, Alès, France; 4Departamento 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 Simón1, Leopoldo Palma1, Alexandra Bernal1, Delia Muñoz2; Trevor Williams3, Primitivo Caballero4;  
1Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutiliva, Spain; 2Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain; 3Instituto de Ecología AC, Xalapa, Mexico
**VI-32** Genome sequence and organization of a *Betabaculovirus* pathogenic to cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae)
Daniel M. P. Ardisson-Araujo; Fernando Lucas Melo; Miguel S. Andrade; William Sihler; Sonia N. Bao; Bergmann M. Ribeiro; Marlinda L. Souza; Laboratory of Baculovirus, Cell Biology Department, University of Brasilia, 70910-900, Brasilia, DF; Brazil. 2. Embrapa Genetic Resources and Biotechnology, Biological Station Park, 70770-917, Brasilia, DF, Brazil.

**VI-33-STU** Analysis of genetic interactions among four non-essential genes of BmNPV Hitomi Taka; Chikako Ono; Masanao Sato; Shin-ichiro Asano; Hisanori Bando; 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, Yasuhsa 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; Delia Muñoz; Jorge Valenzuela; Trevor Williams; 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, Yasuhsa 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.

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**SIP Division Business Meeting:** Wednesday evening

**BACTERIA + Workshop** 20:00-21:30. **P5**

**Non-Target Effects on Biological Pesticides Transgenic Crops**
Moderator: Ken Narva

**199** The impact of herbicide tolerant crops on non-target organisms Ramon Albañés; Marina S. Lee; and Aigrès 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, Projekträger Jülich, Jülich, Germany
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,2,3, Kevin Fitzsimmons1 and Donald V. Lightner1, AQuaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ 85721, USA; 1Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ 85721, USA; 2Department of Aquaculture Pathology, Nong Lam University at Ho Chi Minh, Vietnam; 3Department of Aquaculture Pathology, Nong Lam University, Ho Chi Minh, Vietnam

9:00 205 The Next Generation of Crustacean Health: Disease Diagnostics Using Modern Transcriptomics K. Fraser Clark1,2,3; Spencer J. Greenwood2,3; Atlantic Veterinary College Lobster Science Centre; 1Centre for Environmental Sciences, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada; 2Department 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 Hartikainen1,2, Grant D. Stentiford1,2; Kelly Bateman1,2; Stephen W. Feist1,2; David M. Stone1,2, Matt Longshaw1,2; Georgia Ward1; Charlotte Wood1; Beth Okamura1 and David Bass1, 1Department of Life Sciences, The Natural History Museum, London, UK; 2European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset, UK; 3Aquatic Pests and Pathogens Group, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset, UK; 4Fish Vet Group, Inverness; 5ETH Zürich and Eawag, Duebendorf, Switzerland

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

8:15 208 Insecticidal activity of Heterorhabditis bacteriophora Shandong toward Brontispa longissima and Cryptothele variegata Cheng Bai1, Liping Liu, Haibong Long, Qian Jin and Zhigang 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.

9:30 209 Prospects for using Entomopathogenic Nematodes to Control the Vine Mealybug, Planococcus ficus, in South African Vineyards Patrice D. Le Vieux, Antoinette P. Malan; Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, Matelide, South Africa.

9:45 210 New data on Steinernema ichnusae distribution in the Mediterranean Area E. Tarasco1; M. Causi2; G. Rappazzo2, M. Oreste1, L. Rubino2, D. Leone2, M. T. Vinciguerra2; 1Department of Soil, Plant and Food Sciences; Section of Entomology and Zoology, University of Bari “Aldo Moro”; Bari (Italy); 2Department 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 Torrini1, Beatrice Carletti1, Giuseppe Mazza1, Pio Federico Roveri2, Elena Fanelli3, Francesca De Luca4, Alberto Troccoli5, Eustachio Tarasco2; 1Agricultural Research Council - Agrobiology and Pedology Research Centre (CRA-ABP), Firenze (Italy); 2Istitute of Plant Protection (IPP)-CNR, Bari (Italy); 3Department 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 Rall-Udo Ehlers, e-nema GmbH, Schwentinenthal, Germany

9:45 214-STU Perspectives of new nematode formulation technology for biological control to pest insects in Georgia Mariam Chubinishvili, Tsissia Chkhubianishvili, Manana Kakhadze, Jatamze Malania, Kanchaveli Institute of Plant Protection, Agricultural University of Georgia, Tbilisi, Georgia
Contributed Papers  
Thursday, 8:00-10:00.  
P1  

Viruses 6  
Moderator: Ady Abd-Alla and Madoka Nakai

8:00  

215 Interactions between salivary gland hypertrophy virus and tsetse microbiota Güler Demirbaş Uzel, Vangelis Doudoumis, Antonios Augustinos, Gisele Ouedrogo, Andrew Parker, Drion Boucias, Kostas Bourzis, Ady Abd-Alla. 1Insect Pest Control Laboratory, Joint FAO/TAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria; 2Department of Environmental and Natural Resources Management, University of Patras, Agrinio, Greece; 3Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

216 STU Mechanisms of tree-top disease induced by the specialist baculovirus SeMPV Yue Han, Stineke van Houte, Vera I.D. Ros, Just M. Viak and Monique M. van Oers. 1Laboratory of Virology, Wageningen University, Netherlands

217 Temporal proteomics to study virus infection and function in the host cell Iklai Anag Ince, Sjef Boeren, Just Viak, Monique van Oers. 1Department of Medical Microbiology, Acbadem University, School of Medicine, Istanbul, Turkey; 2Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands; 3Laboratory of Virology, Wageningen University, Wageningen, The Netherlands

218 Characterization of an atypical fast-killing ascovirus: Spodoptera frigida ascovirus 1d (SFAV-1d) Eiko Arai, Shiori Sagawa, Yasumasa Saito, Xiao-Wen Cheng, Dennis Bideshi, Maki Inoue, Yasuhisa Kunimi, Brian Federico, Madoka Nakai. 1Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; 2Department of Microbiology, Miami University, Oxford, Ohio, USA; 3Department of Entomology, University of California, Riverside, USA; 4California Baptist University, Riverside California, USA

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

9:00  

220 Mechanism underlying virus-induced hyperactive behavior: Substrate identification of the baculovirus protein tyrosine phosphatase Stineke van Houte, Carmen Embregs, Esther van Andel, Vera I.D. Ros, Just M. Viak and Monique M. van Oers. 1Laboratory of Virology, Wageningen University, Wageningen, Netherlands

9:15  

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, Bergmann Moráis Ribeiro, Fernando Lucas Melo. 1University of Brasilia - UnB - Brazil

9:45  

222 The essential baculovirus protein VP1054 is a hijacked cellular PURA, a nucleic-acid-binding protein specific for GGN repeats Martin Marek, Christophe Romier, Lionel Galibert, Otto-Wilhelm Merten and Monique M. van Oers. 1Biologie Structurale Intégrative, Institut de Génétique et Biologie Moléculaire et Cellulaire (IGBMC), UDS, CNRS, INSERM, Illkirch, France; 2Laboratory of Applied Vectoriology, Généthon, Evry, France; 3Laboratory of Virology, Wageningen University, Netherlands
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 Hollenstein1, Joachim Kurtz2, Hinrich Schuleenburg3, Heiko Liesegang1, 1Georg-August University Göttingen, Institute für Mikrobiologie und Genetik, Germany; 2Westfälische Wilhelms-Universität Münster, Germany; 3Christian-Albrechts-Universität Kiel, Zoological Institute, Germany

10:00-10:30 BREAK

SOCIOITY FOR INVERTEBRATE PATHOLOGY  
Annual Business Meeting  
Presiding: Jürgen Eilenberg

12:30–14:00 LUNCH  
Mensa

Symposium 8 (Cross-Divisional)  
Thursday, 14:00-16:00.  
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 Lerer, 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 Rolf, 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 Vanhove1, Annick Jacq2, Frédérique Le Roux2, Tristan Rubio1, Alexandre Calteau1, Evelyne Bachère1, Julie Nicod1, Agnès Vergnes1, Astrid Lemire1, Guillaume Chanière1 and Delphine Destriramet-Garzen1, 1Ecology of coastal marine systems, University of Montpellier, France; 2Institut de Génétique et Microbiologie, Université de Paris Sud, France; 3Integrative Biology of Marine Models, Ifremer, Université Pierre et Marie Curie. Station Biologique de Roscoff, France; 4Laboratory of Bioinformatics Analyses for Genomics and Metabolism, Genoscope, Evry, France

14:00 235 Establishing the fungal entomopathogen Beauveria bassiana (Ascomycota: Hypocreales) as an entophyte in cucurbits for managing Zucchini Yellow Mosaic Virus (ZYMV)  
Lara P. Jabar & 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 Hypocre a lili isi acquires protection against Liriomyza huidobrensis (Diptera: Agromyzidae) in the field  

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 entomopathogenic interactions, Surendra K. Dara, Division of Agriculture and Natural Resources, University of California

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 Bass1, Hanna Hartikainen2, Cedric Berney1, Sigrid Neuhauser1, Georgia Ward3, Grant Stentiford4; 1Division of Genomics and Microbial Diversity, Department of Life Sciences, Natural History Museum, UK; 2ETH Zürich and Eawag, Duebendorf, Switzerland; 3European 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  
Kristina M. Crava, Agata Jakubowska, Salvador Herrero, Baltasar Escriche, Yolanda Bel. Department of Genetics, ERI BIOTECMED, Universitat de Valencia, Burjassot, Spain
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<td>14:00</td>
<td><strong>Lysine Residues in N-terminal Tail of a Viral Histone H4 are Crucial in Controlling Host Gene Expression</strong></td>
<td>P1</td>
<td>Rahul Hepat, Yonggyun Kim, Department of Bioresource Sciences, Andong National University, Andong, Korea</td>
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<td>14:15</td>
<td><strong>Heat-shock protein 90 is a broadly active regulator for baculovirus infection</strong></td>
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<td>Shufen Li, Dianhai Hou, Fei Deng, Hualin Wang, Manli Wang, Zhihong Hu, Wuhan Institute of Virology, Chinese Academy of Sciences, P. R. China</td>
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<td><strong>Development and immunity-related microRNAs of the lepidopteran model host Galleria mellonella</strong></td>
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<td>Krishnendu Mukherjee and Andreas Vilcinskas, Fraunhofer Institute of Molecular Biology and Applied Ecology, Department of Bioresources, Giessen, Germany</td>
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<td>14:45</td>
<td><strong>The sf122 gene of Spodoptera frugiperda nucleopolyhedrovirus modulates key aspects of insect-to-insect transmission and post mortem host liquefaction</strong></td>
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<td>Inés Beperet, Oihane Simón, Trevor Williams, Sarah L. Irons, Leopoldo Palma, Miguel López-Ferber, Linda A. King, Primitivo Caballero, Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutillva, Spain; Instituto de Ecología AC, Xalapa, Mexico; Department of Biological and Medical Sciences, University of Oxford, United Kingdom; LGEI, Ecole de Mines d’Alès, Alès, France; Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain</td>
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<td>15:00</td>
<td><strong>Effect of a Viral Encoded Protein Kinase on Gene Expression in Amsacta moorei Entomopoxvirus Infected Cells</strong></td>
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<td>Hacer Muratoglu, Remziye Nalcacioglu, Basil Arif, Zihni Demirbag, Karadeniz Technical University, Faculty of Sciences, Department of Molecular Biology and Genetic, Trabzon, Turkey; Karadeniz Technical University, Faculty of Sciences, Department of Biology, Trabzon, Turkey; Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada</td>
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<td>15:15</td>
<td><strong>FP25K acts as a negative regulator in the infectivity improvement of AcMNPV Budded viruses</strong></td>
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<td>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</td>
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**IMPORTANT NOTE:** Remove all posters before 18:00

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

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

**SEEING YOU IN VANCOUVER IN 2015**
IMPORTANT NOTES:
These abstracts should not be considered to be publications and should not be cited in print without the author’s permission.

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

**129** indicates abstract number for ORAL presentation.

**B-11** indicates abstract number for POSTER presentation.
In 2003, River Bioscience in South Africa became the first African company to successfully produce and commercialise an insect virus as a biopesticide. 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 nucleopolydovirus (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.

**PSENARY 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) *Photurhus* bacteria for insect control. *Photurhus* 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 RNA-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*, *Photurhus* 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. qnj201301).

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

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

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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.

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

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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 diodeoxyugar ascarylolose 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 bacterivorous 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 IU 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 IUs and is reversible, since temperature-switching IUs 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 ambush 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

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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.

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 zone VOCs in response to insect or nematode infestations.

CONTRIBUTED PAPERS Monday, 14:00-16:00

BACTERIA 1

Discovery of Insecticidal Proteins from Non-Bacillus Bacterial Species

Nasser Yalpani1,2; Dan Altier3, Jennifer Barry3, Jarred Orfl3, Ute Schellenberger4, Adane Negatu3, Scott Diehn1, Virginia Crane1. Gary Sandahl, Joe Zhao5, Dave Cerf5, Claudia Perez Ortega1, Mark Nelson1, Analiza Alves1, Lu Liu6, Gusui Wu1


Address for Correspondence: nasser.yalpani@pioneer.com

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.

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 Wallacott, Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, Missouri 63017

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.

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

Nurcan Albayrak Iskender1; Yaşar Aksu2

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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 RNA gene sequence analysis, the bacterial isolates were identified as Serratia liquefaciens (Tv1), Enterococcus sp. (Tv2), Rhodococcus erythropolis (Tv3), Rhahnella aquatilis (Tv4), Curtobacterium flaccumfaciens (Tv5), Pseudomonas sp. (Tv6). Future research will be tested insecticidal effects of these bacterial isolates against T. viridana..
 Evolution of a Sensor Protein Controlling Production of an Insecticidal Toxin in Plant-Beneficial Pseudomonas protegens

Peter Kupferschmied 1, Maria Péczy-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 Zurich, Switzerland
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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 regulatory protein of the DctB family.

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, 14:45. 12 STU

Two new Bacillus thuringiensis toxins active against Lepidoptera and Coleoptera.

Mikel Domínguez 1, Iñigo Ruiz de Escudero 1,2, Isabel Matas 1,2, Leopoldo Palma 1,2, Delia Muñoz 1,2, Primitivo Caballero 1,2
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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 LC50 of 54 µg/ml, while Bm.1711 protein was active against the lepidopterans Helicoverpa armigera and Ostrinia nubilalis, with an LC50 of 164 and 34 ng/cm2, 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, 15:15. 14 STU

Entomopathogenic Bacillus thuringiensis as PGPR

Jiaheling Qi 1,2, Daigo Aiuchi 1,2, Shin-ichiro Asano 1,2, Masanori Koike 1,2
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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 diseases. We already demonstrated that the antagonistic activity of B. thuringiensis AS17 japonensis, AS18 kurstaki against Fusarium oxysporum f.sp. lycopersici 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?

Carmen Lopez-Joven 1, Jean-Luc Rolland 1, *, Eric Abadie 2, Mohamed Laabir 1, Estelle Masseret 1, Audrey Vanhove 1, Audrey Caro 1, Delphine Bonnet 1, Delphine Destoumieux-Garzon 1

1 Ecological of coastal marine systems, UMR 5119, CNRS, Ifremer, IRD, University of Montpellier, France; 2 Laboratoire Environnement Ressource du Languedoc Roussillon, Ifremer, Sète, France.

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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 plankton 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 >180 μm plankton fractions, respectively. *V. aestuarianus* was also detected in fraction 5-180 μm in May and >180 μm 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* genus as well as with microalgae of the *Alexandrium* genus. 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.

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, particles that have the ability to colonize the plant roots and either associate to recurrent mortalities of oysters. However, to date, Vibrios cause major losses in shellfish farming and are associated to plankton species. What possible consequences on pathogen transmission to oysters?

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

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Contributed paper. Monday, 14:00. 17

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

Tyler A. Garretson and Xiao-Wen Chen

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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 H9 and Sf9 cell infection through confocal microscopy. During late phase of infection, small aggregated FP25K-EGFP was found expressing 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  
*Laila Gasmi*, Agata K. Jakubowska, Juan Ferré, Salvador Herrero  
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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* nucleopolyhedrovirus (BmNPV) into BmN Cells by Macropinocytic Endocytosis  
Jinshan Huang1,2, Bifang Hao1,2, Chen Cheng1, Fei Liang1, Xingjia Shen1,2  
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Abstract: *Bombyx mori* nucleopolyhedrovirus (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-dependent endocytosis pathway, but actin, microtub-, PKC-, Rac1- and PI(3)K-dependent, 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 alphanhelical domain aa107-133. However alanine mutagenesis of some of the basic residues (E121A, R122A, K126A and even double E121A/R122A) predicted to destroy the alpha helix, 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 dna pol 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

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The nucleocapsids (NC) of alphacoculoviruses 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 BV virions. 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 chromosome 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 dsDNA cleavage, hence polymerizable actin is also required. An SV40-based model of baculovirus replication will be presented.

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

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The Twist in Baculoviruses

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It has been known 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 chromosome 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 dsDNA cleavage, hence polymerizable actin is also required. An SV40-based model of baculovirus replication will be presented.
(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^2 of leaf area in the citrus canopy caused total mortality ranging from 60-95%. 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**

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During autumn-winter 2012 was conducted a field trial with applications of biorational products B. thuringiensis (Versa™), Pyrethrinis (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, Mexico. The variables evaluated were larvae mortality (LM), fruit damage and yield. The better treatments were: B. thuringensis 39.6%, Pyrethrinis 32.3% and S. carpocapsae 23.3%, while the native B. bassiana strains (2.1x10^6 spores/ml) had 6.3 to 6.6%, and the control 2.6% 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 desiccated microsclerotia (Mb, MS) granules into hydromulch [a mixture of water + wheat straw mulch + pyshium 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 overwinter period, total beetle fecundity was highest in high-humidity controls, females produced 18.5 viable offspring compared to high-humidity hydromulch treatments that significantly reducing 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<0.0001). Importantly, rainfall was significantly correlated (P<0.0002) 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 larva 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 conidia 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 whereas were alive after 3 days with fluazinam applied. 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.
Use of Beauveria bassiana (Bals.) in the management of larger grain borers, Prostheopatus 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 (RGB), Prostheopatus 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 into the country in the early 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, 15:00. 29

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

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Western flower thrips (WFT), Frankliniella occidentalis, is a major pest of ornamentals. Mycotoxized 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. Mycotoxized 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-infection, 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.

Contributed paper. Monday, 15:15. 30

Microsporidiology: Advances in Europe

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

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 M. daphniae, is remarkable for its extreme parasitism on its host, mitochondrial, 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.

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

**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 metchnikovellid parasites from polychaetes *Pygospio elegans* sampled in the White Sea silt littoral zone. The first species, *Metchnikovella incurvata*, Caulfield 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 metchnikovellids and other microsporidia, I believe that the following traits could be treated as plesiomorphic among microsporidia: paired nuclei, meiosis, division by internal budding (endopygeny), short or anisofilar polar filaments, and sequence producing thick-walled environmental cysts. Metchnikovellid spores possess short polar filaments (manubria) and likely do exploit the mechanism of dispersion via evertion of the polar tube with the attached sporoplasm, the major synapomorphy of Microsporidia. At the same time metchnikovellid sporonts 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 metchnikovellid may allow dissemination only within one cell (autoinvasion), whereas production of thick-walled cysts enables horizontal transmission of spores among hosts.

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

**Parasite takes fly - A Drosophila model of Microsporidia infection**

**Sebastian Niehus** 1, **Adrien Franchet** 1, **Frédéric Delbac** 2, **Michael Boutros** 3, **Dominique Ferrandon** 1

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**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 compiled by experts including evolutionary and molecular biologists, veterinarians, entomologists, immunologists 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 microsporidologists 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 assembly in Swiss wheat and maize cultivation
Raquel Campos-Herrera1, Geoffrey Jafuel1, Xavier Chiriboga1, Rubén Blanco-Pérez2, Marie Fesselet2, Vladimir Půža3, Fabio Mascher1, Ted C. J. Turlings1
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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 Acroboloides -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 EPNs 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 how 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 LT50 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 10^5 cells ml^(-1) of *Xenorhabdus bovienii* and *X. canabiliensis* for *S. feltiae* and *S. riobrave*, respectively, in semi-fluid nematode growth gelite. 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 x10^5 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_0*) (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 *S. riobrave*.

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**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 (*Anomaia 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)-8-caryophyllene is more attractive than pregeijerene.

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**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/S0002245914000194).

Morphometric analysis of morphological traits commonly used in nematode taxonomy (referred 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.

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**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.
**EAT OR BE EATEN: FUNGUS AND NEMATODE SWITCH OFF AS PREDATOR AND PREY**

42 STU

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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 fung. 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**


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 phyloplane bacteria in the parental generation and measured the impact on immunity and resistance to NPV in the offspring. The treatments, particularly the follicar 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.
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 losses 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.

Contributed paper. Monday, 17:15. 46

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 homogeneous 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, 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 successive cropping cycles. The aim of this work was to study the efficiency of using mixtures of two SeMNPVs 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 (LC50) 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 genotype 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 F1 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.
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 (5th 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.

**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 SIMNPV, 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 betaculovirus (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 x 10^7 OB/mL to 1 x 10^8 OB/mL. For each mixture, the median lethal concentration (LC50) 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 LC50 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.

**CONTRIBUTED PAPERS Monday, 16:30-18:00**

**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 brongniartii* (3), *Beauveria amorpha* (2), *Beauveria vermiconia* (2), *Beauveria asiatica*, *Beauveria australis*, *Beauveria kipukae*, *Beauveria malawiensis*, *Beauveria sungii* and *Beauveria varioae*. 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, antioxidant, UV tolerance and virulence of Beauveria bassiana**

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The biocatalytic 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 damage to most biomolecules such as DNA, protein, and lipids. Fungal superoxide dismutases (SODs) that detoxify superoxide anions could be putative virulence factors for entomopathogenic 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*BbSod5*. Δ*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 H2O2 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 15-35°C, with optimum temperature ranging from 23.8-28.7 °C. All isolates showed maximum germination values between 1 and 0.996 water activity (aω). Germination at aω 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, 17:30. 56-STU

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

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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 assess 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

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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 EF could be a long and expensive process, the RABFCA-REBECA decision scheme proposes evaluation of the risks posed by crude extracts. Therefore, three fungal strains (BIPESOS, 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 of 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 (10¹⁰ 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.
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 can be separated by 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 kr2 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 mannoside 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 ΔBbktr1/ktr1, ΔBbktr4/ktr4, ΔBbkre2/kr2 and wild-type were constructed. ΔBbktr4 and ΔBbkre2 grew 50–83% slower on nutrition-rich and limited media while ΔBbktr1 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 osmolality, oxidation, wet heat and UV-B irradiation during colony growth. Furthermore, the ΔKtrp mutants were altered in cell wall structure and composition, which contributed to the thickness of cell wall, increased sensitivity to lyase, the low conidial hydropobicity 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 ΔBbktr4, ΔBbkre2 for 18% and 1.2-fold with topical application, and 31% and 26% with intrahemocoele injection. Our findings revealed that Ktrp plays a central regulatory role in B. bassiana.

Theory and Hypothesis: 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 m², 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 CO₂. 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 CO₂ 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 CO₂-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 3 (Fungi) Tuesday, 8:00-10:00

Symposium. Tuesday, 8:00. 58

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

Conifer - bark beetle - fungus interactions
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Tree-killing bark beetles such as the spruce bark beetle (lps 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 both innate 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 monoterpens. 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.
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 disc without spores, while P. persimilis spent less time feeding on the leaf disc 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.

How Fusarium graminearum influences insect-plant interactions

Draulic Jassy, Bruce Toby, Ray Rumiana.

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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 inflected 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 environment created by the pathogen.
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 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 that 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 diffuson discs soaked in 5 μl of 50, 100, 500, an 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.
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 mature 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.
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
Huachao Huang¹, Manli Wang¹, Xin Luo¹, Xi Wang¹, Basil M. Arif², Fei Deng³, Hualin Wang³, Zhihong Hu⁴
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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 nucleopolyhedrovirus (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 disulphide 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 (PhopGV)
Michael D. Jukes¹, Caroline M. Knox¹, Sean D. Moore² & Martin P. Hill³
¹Department of Biochemistry and Microbiology, P.O. Box 94, Rhodes University, Grahamstown, 6140 South Africa, ³Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown, 6140 South Africa

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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
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* and *egt* genes confirmed the identity of the virus as PlxyGV. Phylogenetic analysis of egt sequences grouped PlxyGV-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.

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 *lef8*, *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 virus in both the field and storage.

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

**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*, *ecdysonoid 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. AF27987.1) 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 $3.56 \times 10^6$ 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.**

**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-M122 was followed by qPCR to prove 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 (*lef*-1, *mcp*, *pe-38*, *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.**

**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 *SMNPV* 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: *SMNPV* 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.
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 fields. 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 californica multiple nucleopolyhedrovirus (AcMNPV) PEP, such as Helicoverpa armigera nucleopolyhedrovirus PEP, Cydia pomonella granulovirus Cp20 or Cp22 might not repair the absence of polyhedron envelope in the pep-knockout AcMNPV construct. The C-terminal (168-252aa) of AcMNPV PEP might deliver 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.

BACTERIA 2

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. Marshall1, Jason N. Busby2, J. Shaun Lott2, Sandra A. Jones2, Julie E. Daiziel1, Femke Schepers2, Mark Hurst1, 1Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch 8140, New Zealand; 2School of Biological Sciences, University of Auckland, Auckland 1142, New Zealand; 3Food & Bio-based Products, AgResearch, Grasslands Research Centre, Palmerston North 4442, New Zealand
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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 (Ch1 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 Photobahbus luminescens. Characterization of Yen-Tc pathogenesis 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 Segond1,2, Agnès Rejass1, Christophe Buisson1, Shuyuan Guo1,2, Karine Adel-Patient1,4, Hervé Bernard1,4, Didier Leruclus1, Christina Nielsen-LeRoux1, 1INRA UMR1319-Micalis, team GME, 78352 Jouy en Josas France, 2INRA, UR496 Unité d’Immuno-Allergie, Institut Pasteur, 78352 Jouy en Josas, France, 3School of Life Science, Beijing Institute of Technology, 100081, Beijing, China, 4CEA, IBiTecs, Service de Pharmacologie et d’Immunanalyse, F-91191 Gil-sur-Yvette, France
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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.
mor ABC transporter C2 (BmABCC2) functions as a receptor for Cry1A toxins. We also demonstrated that BmABCC2 can confer approximately 10-1000 times higher susceptibility to the cells than cadherin-like receptor (BIR175) and BmABCC2 and BIR175 co-expression exerts synergistic effect in susceptibility conferring ability. This synergistic effect suggested that these two receptors have different roles in the mode of action of Cry1A toxins in S9. 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 continuously increased during toxin incubation, indicating that pores were continuously assembled on the cell membrane. However, when BIR175 expressing oocytes were administrated with toxins current increment speed was lower than in BmABCC2 expressing oocytes, indicating that BIR175 has lower function than BmABCC2 in pore formation helping. In contrast, BmABCC2 and BIR175 co-expressing oocytes showed at least 4 times higher current increment speed than BmABCC2 single expressing oocytes. This indicates that synergism occurs at least in part in the pore formation process, although synergistic effect is very low in comparison to that seen in S9 expression system. We also compared the binding affinity to Cry1A toxins of BmABCC2 and BIR175 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 cytotoxic 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 cytotoxic 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 STU

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 nematocidal 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 Dicer-1/Dicer in C. elegans antibacterial immunity as well as identified novel nematicidal toxins.
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 Aedine 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 Aedine vector species may provide robustness to the primary siRNA-based antiviral response.

Controlling viral infection 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.

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
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 1x10⁸ 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
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, 11:45. 97-STU
Wireworm control with entomopathogenic fungi and plant extracts
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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 density 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 occurring strains. Highest densities of Beauveria spp. (up to 6.8 x 10^7 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
PRES. OF BENEFICIAL INVERTEBRATES

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, 10:30. 99
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 haemaphila

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Contributed paper. Tuesday, 10:45. 100

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

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

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

Lauren Hall1, Chris Hauton1, Grant Stentiford2
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Contributed paper. Tuesday, 11:15. 102-STU

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

Amanda Smith, Andrew Rowley
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Contributed paper. Tuesday, 11:30. 103-STU

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 influence 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.
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 panzoonic 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 predated 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 penaeid shrimp. Dendronereis sp. is a most ubiquitous resident annelid in shrimp ponds and used as food source for shrimp. We showed that WSSV replicates in Dendronereis sp. and can be transmitted from this polychaete to penaeid shrimp. Furthermore there appears to be a positive correlation between the last incidence of WSD in ponds and the occurrence of WSSV in resident Dendronereis sp. There is also correlation with other pond parameters. We hypothesize that Dendronereis sp., 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, 11:45. 104

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 glycan 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 lipopolysaccharides (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, 12:00. 105

Fungi 3

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

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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 sparsoprobolasts with 8 spores (3.6 x 2.1µm) and anisofilar (2+6) polar filaments, the features corresponding to the diagnosis of Agmasoma penaei (=Theohania 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, 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 >95%) LA isolate was identical to Perezia nelsoni from the Mississippi 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 paper. Tuesday, 10:30. 107
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 conidial 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 *Pseudococcus 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, Pyralidae), – *Habrobracon hebetor* (Hymenoptera, 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 mycosis of the native host and conidia formation. 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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108-STU

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

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**Contributed paper. Tuesday, 11:30. 111**

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**Contributed paper. Tuesday, 11:45. 112**

**Simon Tragust1, Heike Feldhaa, Jes Søe Pedersen2**

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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 epizooic 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 entomopathromycota-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 entomopathogenic 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 transmitting 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. Furthermore, E. formosa was able to 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.

Contributed paper. Tuesday, 12:30. 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 developments/Issues in the Regulatio
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
Jarrod Leland
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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
Sabine Asser-Kaiser, Jacqueline Süß, Rüdiger Hauschild
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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

Maria Helena N. L. Silva-Filha, Karlos D. M. Chalegre, Tatiana P. Romão, Daniella A. Tavares, Hervely S. G. Menezes, Cláudia M. F. de Oliveira, Osvaldo P. de-Melo-Neto

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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 homologous larvae for each one, through different approaches. The alleles named *cqm1REC1* and *cqm1REC2* 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 *cqm1REC2* has predominated during a long period (> 100 generations), however, it has been replaced by the *cqm1REC1* that became the most frequent allele. A colony established from the cross of homozygous individuals from each allele (1:1 ratio) showed that *cqm1REC1* assumed a higher frequency, compared to *cqm1REC2* during a period of 21 generations. An AS-PCR-screening detected the presence of *cqm1REC2* allele in larvae from field populations and its frequency and distribution was lower than that found for *cqm1REC1* 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

Pascale Flury, Beat Ruffner, Shakira Fataar, Maria Pechy-Tarr, Regina G. Kleespies, Cornelia Ullrich, Johannes A. Jehle, Theo H. M. Smits, Christoph Keel, Monika Maurhofer

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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 S19 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*. 
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, withstandings 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 infection, there were three mutant alleles. Although this type of transposon is typically lethal at the transcriptional level, but in a different location. Allele frequency changes were recorded over several generations of artificial selection in the laboratory.  Although this type of transposon is usually lethal at the transcriptional level, but in a different location. Allele frequency changes were recorded over several generations of artificial selection in the laboratory. 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 the insertion of a transposable element, but in a different location. Allele frequency changes were recorded over several generations of artificial selection for a homoyogous 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 cotton expressing Cry1A toxins.

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 the insertion of a transposable element, but in a different location. Allele frequency changes were recorded over several generations of artificial selection for a homoyogous 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 cotton expressing Cry1A 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 IC50 than _N. apis_. The effect is most pronounced at 1 day post coclosion. 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 epithelial cells of the midgut and elicit nosomosis. 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 andZW 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.

**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.

**A first report of an immune-associated cytosolic PLA2 in insects: Gene structure and function**

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Eicosanoids are a group of C20 polysaturated fatty acids most derived from arachidonic acid (AA). A phospholipase A2 (PLA2) catalyses AA release from phospholipids at SN-2 position. Among three different groups of PLA2s (cPLA2, sPLA2, iPLA2), only sPLA2 (secretory type of PLAs) has been identified as immune- and immune-associated functions. This study reports the first cPLA2 (cellular and calcium-dependent PLAs) in insects. A hemocyte transcriptome of Spodoptera exigua possessed 1 for sPLA2, 2 for iPLA2, 1 for cPLA2. Expression of Se-cPLA2 was inducible to bacterial challenge in hemocyte and fat body. RNA interference of Se-cPLA2 expression significantly suppressed cellular immune responses of S. exigua. A recombinant of Se-cPLA2 exhibited a specific enzyme activity influenced by pH, temperature, and calcium. Especially, Se-cPLA2 was susceptible to a specific cPLA2 inhibitor, but not to a specific iPLA2 inhibitor. These results indicate that Se-cPLA2 is a specific cPLA2 and associated with immune responses.
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 ABysSS, a total of 10,951 contigs were obtained. These were assembled into 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.
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 similar significance 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, orthologs are shared by entomopathogenic oomycetes and fungi, 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 Aegeolysin 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 1.60 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 Aegeolysin 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 galactosylates 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

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 cytolytic 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 grasp 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 to 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

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 subspp. 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
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/mL1 of dried CFS. The LC50 values were 0.037- and 0.015µg/mL1 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/mL1 CPT for 72 hrs. The LC50 value for A. segetum L1 was 0.417µg/mL1. 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 nematocidal and insecticidal activities against soil-dwelling pests, such as M. incognita and A. segetum.

Contributed paper. Wednesday, 10:45

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 x 108 nematodes per ha obtained equally high control like chemical seed dressings with neonicotinoiodes or application of granular insecticides containing the pyrethroid 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 neonicotinoids 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, 11:15

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 Abdullahman S. Aldawood

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Ten plant extracts were tested as ultraviolet protectants to improve the persistence of Spodoptera littoralis multiple embedded nucleopolyhedrovirus (SpiMNPV). In an initial test, the SpiMNPV 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...
The discovery of Oryctes maimaiga or $O. \text{maimaiga}$, which 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 $S. \text{hortensis}$ incorporated into an ointment. Also toxicity of the liposomal essential oil of $S. \text{hortensis}$ 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 $S. \text{hortensis}$ was determined by GC and GC-MS analysis. The liposomal essential oil of $S. \text{hortensis}$ 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 $S. \text{hortensis}$ incorporated into the ointment and liposomal essential oil formulation have potential antifungal activity against Candida albicans. MTS assay results showed that a concentration of $10^{-7} \mu g$ 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

**Martin A. Erlandson**, 1 B. Cameron Donly, 2

**David A. Thelmann**, 3 Dwayne D. Hegedus, 4 Cathy Coutu 1 and Douglas Baldwin 1

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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

**Aurélien Chateigner**, Cindy Pontieve; Carole Labrousse; Elisabeth Herniou

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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 passing 10 times through 4 different host species, in 10 replicates. We then characterised the genetic makeup of 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

**Germain Chevignon**, Sébastien Cambier; Jean-Michel Drezen; Elisabeth Huguet; Sébastien Moreau

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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 Polydnaviruses (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 catterpillar 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 scale transcriptomes of non-infected and parasitized *M. sexta* larvae. Following this analysis, we were able to identify 76 *CcBV* genes and 1993 *M. sexta* genes expressed 24hrs after parasitism.

**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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We conducted a large scale transcriptomic analysis of *M. sexta* infected by *C. congregata* using a union of four complementary datasets covering the total depth of 1.9 millions reads (total sequenced of 1.2 Gb). We identified 592 up-regulated and 845 down-regulated *CcBV* ORFs in parasitized vs. non-parasitized larvae. We identified one *CcBV* transcript expressed exclusively during parasitism. 30% of *CcBV* ORFs encode proteins involved in host infection and host immune evasion. 15% of them are new and 30% of viral genes are new targets for molecular and functional studies.

Using a reverse approach we isolated new baculoviruses from *M. sexta* infected by *C. congregata*. We isolated three baculovirus-like particles (BVP) masses by latex agglutination from infected larvae. The BVP masses were sequenced using 454 pyrosequencing and compared with previously published and registered nucleopolyhedrovirus (NPV) and nucleocapsid polyhedrovirus (NPV) genomes. Two of the BVP masses showed high similarity with previously published NPV genomes, however one BVP mass (~45 kb) is new and closely related to AcMNPV. We named this virus *C. congregata* nucelopolyhedrovirus (*CcNPV*).

*CcNPV* will be the first NPV species of the Hymenoptera parasitic at larval stage. The genome of *CcNPV* is composed of multiple fragments that are assembled into a single linear molecule. The genome size of *CcNPV* (~48 kb) is one of the smallest baculovirus genome identified. Its gene content (119 ORFs) is also unique among baculovirus genomes. *CcNPV* possesses a high rate of gene duplication and atypical intron distribution.
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.

Population genomics supports baculovirus vectors as agents 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.

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 Massachusetts, 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 ORF id31, id66, and id133 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 (id70) and id76 and between id111 and id112 (id116). A 2-n deletion in the enhancer gene vef-2 (id160) 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 (id65). 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.

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 LdMNPV isolates from North America, Europe, and Asia.
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.

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 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..

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 × 10⁷ 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 × 10⁷ 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.

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), and the moth *Paysandisia archon* (Burmester) (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* (RI) 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* EFPs 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 EFPs strains as function of their genealogy and geographic origin. Finally, virulence of selected isolates was tested against both RI 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, 10:30-12:15

Fungi 5

**CONTRIBUTED PAPERS Wednesday, 10:30-12:15**

**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 × 10⁷ 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 × 10⁷ 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

**Interactions between the insect pathogenic fungi Metarhizium, the wheat pathogen Fusarium culmorum and the mycoparasitic fungus Clonostachys rosea**

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The wheat pathogen *Fusarium culmorum* 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 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.
The prospect of utilizing 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) 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-a 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.

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. Hypocrean 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.

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 (Trichodermia 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, β-
cymene, α-Terpincenol, β-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**

**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 have the potential to 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**

**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 bipunctata* L., and convergent lady beetles, *Hippodamia convergens* Guérin-Méneville 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. hippocampi* and *N. adaliae*) are transmitted to green lacewings and to examine the effects of these pathogens on lacewing larvae and adults.

**Contributed paper. Wednesday, 14:45**

**Multi-genie phylogeny applied to the taxonomy of microsporidian parasites of crustacean hosts**

K.S. Bateman1, R. Ken1, D. Wriedu-Boakye2, B. Williams2, G.D. Stentiford1

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Hepatospora, 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 (>89% 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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Enteroctozen 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 A. mellifera 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. We offer experimental data supporting the identities of NcORF01663 as polar tube proteins (PTP) 1 and 2 and NcORF-01664 and NcORF-01663 as polar tube proteins (PTP) 1 and 2

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We offer experimental data supporting the identities of NcORF01663 as polar tube proteins (PTP) 1 and 2 and 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.

**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 *Anncalia 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.

**Contribution:** Wednesday, 15:45 **176**

**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 EEPf, 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.

**Contribution:** Wednesday, 14:30 **179-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 *C. quinquefasciatus*. The characterization studies confirmed the spherical shape and size (3–24 nm) of silver nanoparticles. The efficacy of fungus AgNPs tested concentrations of 25 and 50 ppm against L1, L2, L3 and L4 instar larvae of *C. quinquefasciatus*. The LC50 (LC90) 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, LC90 (LC50) 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 instars of larvae of *C. quinquefasciatus*. These finding use of fungus synthesizes silver nano-particles is a rapid, eco-friendly, and a single-step approach and potential mosquito larvicidal agents.

**Contribution:** Wednesday, 14:15 **178-STU**

**Pathogenicity of three entomopathogenic fungi on larvae and adults of the silkworm: The silis less the better?**

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**Contribution:** Wednesday, 14:45 **180**

**Microbial Control 3**

**Contribution:** Wednesday, 14:15 **179-STU**
The sisle weevil was first recorded in Greece on May 2010 on ornamental plants of Agave sp. As the use of synthetic pesticides 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 Rhyynchophorus ferruginus 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 effectiveness of application through treated surface. Beauveria bassiana and M. anisopliae were applied in concentrations of 10^2 and 10^3 conidia/ml while I. fumosorosea was applied at a concentration of 10^4 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±5.3%). 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

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, hemocentin 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 hemocentin, 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

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 preimaginals. 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 (Glyphosate ) and 3.3 mm d^-1 (Oxyfluorfen). This compatibility was also revealed in vivo by assaying the fungus towards medfly prepatrating larvae in herbicide containing soil (1.0 x 10^3 conidia g soil^-1). Even if there was a decrease of the M. brunneum level until 10^-15 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

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.
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 (Dioctria virgiferaeformis) 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 virginiana*. 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 horrida* 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.

**Soybean aphid viruses exploit contrasting transmission strategies**

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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 (Epirocta). 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.

**Discovery of circular single-stranded DNA viruses in top insect predators**

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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 specimens homogenates through filtration, DNA was then

**Single-stranded DNA viruses in marine crustaceans**

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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.

### 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, Paroviridae, 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 uninterrupt ed 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 known viruses from members of the Densovirus, Circovirus, Nimaviridae, Paroviridae, and Totiviridae 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 denovirus-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, denovirus-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 paroviruses 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 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 endosymbiotic 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.

BACTERIA 4

Contributed paper. Wednesday, 14:00 192

Analysis of the bacterial community of the insect pest Lymnaea dispar during its life cycle

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Gypsy moth (Lymnaea 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.
Interactions between the Med fly Coratillis capitata (Wied.)and a new Bacillus cereus sensu lato strain
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The Med fly Coratillis 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, aldehydeg dehydrogenases, peptides 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 vulnificus 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.

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. Power biopesticide was produced using this cell suspension and suspension was produced in pilot fermenter. Powder biopesticide was supported in synthetic formulation materials in the spray dryer. The physical and biological properties like wettability, susceptibility, 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 Stophillus granarius adults in laboratory conditions were investigated. Mortality results were identified as 37% against S. granarius and 100% against Agelastica alni.

The taxonomic genus Rickettsiella (Gammmaproteobacteria; Legionellales) comprises intracellular bacteria associated with a wide range of arthropods including insects, arachnids and crustaceans. Ultrastructurally 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 known 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.


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*.

**Pseudomonas protegens**

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The root-colonizing biocontrol agent *Pseudomonas protegens* CHA0 protects its plant hosts from fungal diseases by releasing toxic exoproteins 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 ΔfitG ΔfitH* 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.

**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 to be the most effective with a wide range of activity against insect-pest species. 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.

**Bacillus thuringiensis**

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A novel *Bt* strain, *TK-E6*, isolated from groove 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 cry1A promoter and cry4A terminator. Each Cry protein was purified and used for mosquitocidal assay against *Ae. aegypti* larvae, 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 live...
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 LC50, Cry1Ac was the most active protein, whereas the rest of proteins were similarly active. However, at the level of LC90, 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 LC90 than at the LC50 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 125I-radio labeling.**
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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 125I-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.

**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.

**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 in Bt resistant insects.
(PxCad), 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.

RNA interference of integrin subunit β1 impairs development and immune responses of the oriental tobacco budworm, *Helicoverpa assulta* against bacteria

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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* Cry2Aa 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.

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 rRNA base 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 loops, 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 intra-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. melilotthae' 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. melilotthae' PmrAB drives expression from the promoter regions of the presumed homologous T4SS gene clusters.

Comprehensive in silico analysis of PmrAB regulon 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
Pseuobacillus larvae and the virulence factor SplA: an ERIC II specific S-layer Protein
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Pseuobacillus 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 splA-gene was successfully interrupted. In infection assays, SplA-deficient P. larvae and the parental wild-type bacteria were compared and it was demonstrated that lack of SplA expression resulted in a significant decrease in total mortality. To further investigate the role of SplA in virulence of P. larvae, SplA has been expressed in 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
Andrei Papkou, Rebecca Schalkowski, Mike-Christoph Barg, Ines Braker, Hinrich Schulenburg
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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. Paradigmatically, one indispensable and basic property of host-parasite interaction, population size oscillations, has been overlooked as a factor in host-parasite
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-V) 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 antimicrobial 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 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

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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 where *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 a 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.

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 76%, *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-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 76%, *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 Ig-2, Ig-3- and Ig-7- 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 Ig-2-, Ig-3- and Ig-7- 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 Ig-2-, Ig-3- and Ig-7- 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. Herein we present the first haemocyte transcription 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**

David Duval¹, Richard Galinier¹,2, Gabriel Mouahid¹,2, Eve Touilza¹,2, Anne Rognon¹,2, Nathalie Arancibia¹,2, Jean Francois Allienne¹,2, Guillaume Mitta¹,2, André Théron¹,2, Benjamin Gourbal¹,2

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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 systematic investigation has ever been conducted to identify snail microbial pathogen and evaluate its molluscicidal 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**

Natalia A. Kryukova¹, Ekaterina A.Chertkova¹, Alexandra D. Semenova¹, Yuri I.Glazachev², Irina A. Slepenova², Victor V. Glupov¹

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The influents of *Habrobracon hebetor* venom on the cellular and humoral immune reactions of the wax moth larvae (*Galleria*...
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 (AmFV). The life span of bees infected with Nosema and viruses is shorter than of bees infected with Nosema alone. AmFV is a DNA virus. The size of the enveloped particle is 150-450 nm x 150 nm. On the basis of morphological features, AmFV was considered to be related to baculoviruses. In 2012 the first fragment of AmFV 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 AmFV. One of the contigs 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 AmFV will be presented during the conference.

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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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.
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 practitioner 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 maculiferetorum, 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.

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 microsporidian 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.

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 brongnarii, Metarhizium spp., Lecanicillium 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 Lecanicillium
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.

The aim of the BIOCOMES work package is to develop a new pesticide due to resistance to? 

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.

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 "hypomycete" genera, *Beauveria* or *Isaria* (Ascomycota; Hypocreales; Cordycipitaceae). 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.
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 Neocyphites floridana. Pooling all soils samples revealed that Metarhizium spp was the most common fungus (70%-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 / 98.6%-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 (43%-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 boarders as a reservoir of these generalist natural enemies.

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 cycloheximide and SLYLIT 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-1α molecular markers). The two more common taxa were unnamed species designated as Lecanicillium spp. (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.

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.

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 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.
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 with a conidial suspension of *B. bassiana* (Balsam) Vuill with a concentration of 10^7 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 inoculated 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 robertsi* ARSEF 2575 or *M. 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% benomy; air dried; then the fungus suspension was brushed on the leaves. After 24h and 48h, 0.5 cm² 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 (PDA-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 rapeseed 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 napii*). 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* Naturalis ATCC74040 were designed and used for strain-specific diagnosis. While the fungus was found to be persistent on the epidemics, 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**

**Azzygo- 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 azzygospores 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 azYGosporogenesis and azYGosporogenesis was also found in the same individual in the Norwegian strains. 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 matricellular 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 2x10^7 conidia or hyphal bodies/ml of these fungi or treated topically (2x10^7 conidia or hyphal bodies) and then incubated in a permanent water film in a moist chamber at 25°C. 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 was detected 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 molluscidal activity depends on the type of fungal inoculum applied.

**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 of these fungi suggest that these entomopathogenic fungal metabolites might be useful as a source for plant pathogen control and pharmaceutical interests.

**FU-16**

**Evolutionary-ecological strategies of *Metarhizium robertsi***

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The species of the entomopathogenic fungus *Metarhizium* include forms characterized by different pathogenic strategies. Two strains of entomopathogenic fungus *M. robertsi* 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 destructin 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- toxicogenic» strain has higher adaptation to entomoparasitic nutrition, and the «toxicogenic» 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 «toxicogenic» 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.
culture medium incubated at 27±1°C (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 (Twen 80, 0.01%), commercial emulsifiable or non-emulsifiable oils or carboxymethyl-cellulose gel (CMC), and exposed to 45±0.5°C for 4, 6 or 8 h. Germination was assessed 48 h after inoculation of conidia on 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±0.5°C was drastically low [0% mean relative germination (RG) at 8 h 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 8 h 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 (Twen 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 *Metarhizium 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 host. These include the energy-extensive 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*, *Toxoplasma infatum*, *Toxoplasma clyndrosporum*, 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 aleurodis* 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 2500 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 differentially the conidial tolerance to menadione in *Metarhizium robertsi*.  
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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 robertsi* (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 used 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 3 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 Sabouraud-4 glucose - complete medium - and on a modified Czak-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, EMA01/58-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. CM medium, BIPESCO5, ARSEF 23 and EMA01/58-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 synonymized, and their names will no longer be available except in a descriptive manner (e.g., hirsuteloid 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***

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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 producer of toxins and biological active materials, which can cause high mortality in different species of pests. Nowadays, there is high interest towards the study of these fungi from Argentina. *Beauveria* produces toxins and biological active strains isolated from a wide range of habitats and geographical zones of Argentina. Our research aims to identify proteins 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-26**

**Genetic characterization, fungicide sensitivity, and aphidicidal potential of *Lecanicillium* fungis from Argentina**

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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, RP31, RP32 & 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. robertsi* and *M. brunneum*, without the need to obtain full-length sequence reads. Markers included in the recent multilocus phylogeny (ITS, rIGS, EF-1α, EF1-5′, RP31, RP32 and β-tubulin) and 5 nuclear intergenic (nIGS) 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. robertsi* (rIGS) and *M. pingshaense* (nIGS), and two primer pairs were designed for *M. brunneum* (both nIGS). 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 lecani s.l.) by mitochondrial gene sequences**

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For species identification of entomopathogenic fungi of the genus Lecanicillium (former Verticillium lecani Zimm. Vegas) 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 longisorum*. 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**

**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 in rapid mosquito death rather than infection route from tarsomere, and death of mosquito highly correlated with fungal infection 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. 60:3, 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 damage 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 tusciipes*. 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.
The sycamore lace bug, C. ciliata, is one of the most destructive pests of plane trees (Platanus spp.) all over the world. This pest is also known to be major nuisances in Georgia since plane trees have 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 a 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, containing 10⁵ 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 to 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 more tolerant to the induced mycosis caused by I. fumosorosea, but more effect on the mortality of C. ciliata adults. Experiments are needed to determine the IC₅₀ of the fungus for the two pests and to develop appropriate application methods if efficacy proves to be sufficient.

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, Tetranynchus 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. floridana isolate ESALQ1420 placed on coverslips with alphanumeric coded squares. The effect of these pesticides on the sporation 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±189 and 38%±12% of these produced capilliconidia (germinated). RC of Follicur (Tebuconazole) resulted in a primary conidia production of 1658±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%±14) germination and Karate at RC/2 in 2985±337) primary conidia and 83%±0) germination. This demonstrates that Vertimec, Folicur, Danimen, Talcord and Karate (Lambdacyhalothrin) 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±184 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.

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, scarids, 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 bacteriophora), 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 INBIOSOIL 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, ii) 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, 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 alkane, 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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Affordable control of aphids on 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 LT50 values of 2.8, 2.1 and 1.9 d; and the lowest LC50 values of 5.5×10⁵, 8.1×10⁴ and 1.7×10⁴ 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 greenhouse, aqueous and oil formulations of ICIPE 62 significantly reduced aphid population growth rate (r), B. brassicae -0.03 and -0.03 and L. pseudobrassicae -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, 0.03 and 0.01 for A. gossypii, respectively). These results are indicative of the potential of isolate ICIPE 62 in the management of aphids.

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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 submersed 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 1×10⁶ germinable spores were prepared. Five tomato leaves, infected with T. vaporariorum nymphs at 2nd-3rd instars, were submersed 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 submersed spores. L. longisporum was the least affected by the production system. Contrary to Lecanicillium, submersed 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.
Development of entomopathogenic fungi in mosquito control: which kind of production for which efficiency?

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Midges of (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 immersed in a nutrient medium) is a tremendous production system focusing 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 secreted 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.

Selection of entomopathogenic fungi for the control of Aegnorhynus nodipennis (Coleoptera: Curculionidae) under laboratory conditions

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Plum weevil Aegnorhynus 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 Metarthizium anisoplaie and Beauveria bassiana were evaluated on adults of the Plum weevil under laboratory conditions. 45 strains of M. anisoplaie 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 x 10⁶ conidia / insect and mortality was assessed every day for up to 10 days. Two strains of B. bassiana and M. anisoplaie were selected as the most effective on adults. The B. bassiana strain reached 100% mortality and the M. anisoplaie 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.

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 borer 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 tenenbrionis (Btt) from commercial bioinsecticide (Novodor) displayed LC₅₀ and LC₉₅ values of 3.2 and 164 mg g⁻¹ respectively against neonates of Capnodis tenenbrionis, 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.

Susceptibility of Plutella xylostella (L.) (Lepidoptera: Plutellidae) populations to Bacillus thuringiensis strain HD1

Caroline Placidi De Bortoli¹, Ricardo Antônio Polanczyk¹, Neil Cickmore², Rafael Ferreira dos Santos³, Alessandra Marieli Vacari¹ and Sergio Antonio De Bortoli¹
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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

Poster / Microbial Control. Wednesday, 16:30. MC-6

Susceptibility of Plutella xylostella (L.) (Lepidoptera: Plutellidae) populations to Bacillus thuringiensis strain HD1

Caroline Placidi De Bortoli¹, Ricardo Antônio Polanczyk¹, Neil Cickmore², Rafael Ferreira dos Santos³, Alessandra Marieli Vacari¹ and Sergio Antonio De Bortoli¹
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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
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, justified 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.

The Diamondback moth, *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, pupal weight, sex ratio, 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, and 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.

**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 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 *Athleta coriaria* (Coleoptera: Tachynidae), 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 x 10⁷ and 1 x 10⁵ 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.

**Evaluating microbial biocontrol agents: effects of Metarhizium brunneum on non-target arthropod**

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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.

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 10^6 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.

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 oilseed rape. 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.

References

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 1×10^5 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 4.58 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 5150, were selected based on their virulence as well as mycosis/conidiosis for further dose-wise bioassay. Based on the LC₅₀ 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* (BI) 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. brongniarti* 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* Luciana S. Lobo1, Sergio J. Mijailovsky1, M. Patricia Juárez2, Christian Lüüs1, Everton K. K. Fernandes3 and Nicolás Pedrin1
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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 *T. brmg* and *T. 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 *T. brmg* expression, with 1.3±0.5 and 3.0±0.4 fold induction over the controls in insects treated with 1×10^5 and 1×10^7 con/ml, respectively. Similar results were observed for *T. 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. Observe 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 standard methodology - dissecting insect and preparing homogenates. Individua 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 (L.) (Lepidoptera: Plutellidae) is one of the most important pests of crucifer worldwide, and farmers usually control this pest with pesticides that favors the population resistance development, decreasing the population density. Studies on biological control agents such as the entomopathogenic 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, cry2B and cry2Ba but isolates named LCM04, LCM05 and LCM29 amplified gene vip2 and the isolates LCM06, LCM20, LCM4A5 and LCM4A6 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. guildinii* and 95% mortality in *N. viridula*. In the semi-field experiment the *Ma*-30 strain caused the highest mortality and 75% 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: Chrysomelidae), 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 larvae 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* (Eschsclottz) 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* FS2, *Beauveria bassiana* GHA, and *Metarhizium robertsi* 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* (Boisdrua, 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 of S. littoralis larvae (Boisdruval) (Lepidoptera, Noctuidae), which is considered 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 08/88-Su, EABb 10/103-Su, and 3155) and one isolate of Metarhizium caused more than 50% mortality of larvae. EABb 01/33-Su and EABb 01/103-Su and 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 LD50 and LT50 values were 5.69x10^6 conidia ml⁻¹ and 6.76 days for EABb 01/33-Su and 1.05x10⁶ conidia ml⁻¹ and 7.02 days for EABb 01/88-Su. On the other hand, the crude extracts obtained from the isolates EAMa 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 EAMa 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 EAMa 09/01-Su isolate and its extract at 1 mg ml⁻¹ and 76% mortality for EAMa 01/58-Su isolate and its extract at 1 mg ml⁻¹. The combination of the fungus EAMb 09/01-Su at 10⁷ conidia ml⁻¹ and the crude extract had a synergistic effect on larvae resulting in 100% mortality to concentrations 1 mg protein ml⁻¹ and the combination of the fungus EAMb 03/33-Su + extracts EAMb 09/01-Su to concentration of 10⁷ and 10⁶ 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 10⁶ 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 S. littoralis larvae (Boisdruval) (Lepidoptera, Noctuidae), which is considered 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 08/88-Su, EABb 10/103-Su, and 3155) and one isolate of Metarhizium caused more than 50% mortality of larvae. EABb 01/33-Su and EABb 01/103-Su and 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 LD50 and LT50 values were 5.69x10^6 conidia ml⁻¹ and 6.76 days for EABb 01/33-Su and 1.05x10⁶ conidia ml⁻¹ and 7.02 days for EABb 01/88-Su. On the other hand, the crude extracts obtained from the isolates EAMa 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 EAMa 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 EAMa 09/01-Su isolate and its extract at 1 mg ml⁻¹ and 76% mortality for EAMa 01/58-Su isolate and its extract at 1 mg ml⁻¹. The combination of the fungus EAMb 09/01-Su at 10⁷ conidia ml⁻¹ and the crude extract had a synergistic effect on larvae resulting in 100% mortality to concentrations 1 mg protein ml⁻¹ and the combination of the fungus EAMb 03/33-Su + extracts EAMb 09/01-Su to concentration of 10⁷ and 10⁶ 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 10⁶ 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-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 on the beneficial arthropods that are widespread and naturally occurring in Europe and are all also commercially available for biological pest control: Aphidoletes aphidimyza (Insecta, Diptera, Cecidomyiidae), Athela coriaria (Insecta, Coleoptera, Staphylinidae), Onius 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 of 3 × 10⁸ 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 vili present in the midgut of the predator were observed in both cross section and an 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*.

**Microsporidia**

**Poster / Microsporidia. Wednesday, 16:30. MI-1**

**Decline of native bumblebees (Bombus) and Nosema (Microsporidia: 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. bombycina* ITS region for comparison with the European and North American isolates.

**MICROSPORIDIA**

**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 copies.
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 real-time detection of N. ceranae and other microsporidia 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 = log Nt – log Nt-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; r²=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 “Neklinovskiy”, 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 thought 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 xylostaeana, 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 A. xylostaeana 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 N. ceranae 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: Chytridiopoda) from the pigeon feather mite Falculifer rostratus (Asthigmata: 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 poloprast 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 / Microsporida. Wednesday, 16:30. MI-7**

**Infecitivity 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 *phthonandria atrilineata* in the silkworm rearing region of Zhejiang province, China. The mature spores of TMPA were cylindrical or ovocylindrical in shape with a strong dioptr and glossy surface. The spore size of TMPA was 3.27±0.14 x 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 nuclei by meiosis, and later 8 spores were formed in pansporoblast. Infection was systemic with mature spores produced in epithelial cell of trachea, fat body, middle and posterior silkgland, fore and middle intestine, malphigian 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 IC50 value of TMPA to newly-hatched silkworm larvae was 1.55±1.03 spores/ml, 700-fold higher than that of *N. bombycis*, suggesting a weakly infectousness. 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 Sterkelovimermis 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 Argentina. 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. *Sterkelovimermis 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. *Sterkelovimermis 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 JI 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 dry-desiccate in an environmental chamber set at 23°C 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 (p<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.
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-kil 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.

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 nematophilus*. 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.

Our research was supported by the project KONTAKT II LH14047 and program C2.1.07/2.3.00/30.009 co-financed from European Social Fund and the state budget of the Czech Republic.

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 *Otiorychus sulcatus*, Plum weevil *Aegorhinus nodipes* and Raspberry weevil *Aegorhinus superciliosus*. The most effective NEPs have been isolated of *Steinernema australae*, *S. feltiae* and *S. unicomum*. 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-5

Nematodes of large larch bark beetle *lps 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 intestinal system 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 *Micoletzya*. In haemocel adult females and juveniles of *Contortylenchus* sp., *Parasytlenchus* sp. and members of *Cryptaphelenchus* sp. were found. While in intestine the juveniles of *Parasitohabditis* sp. and some tylenchid juveniles were found too. The large larch bark beetle gallery content was examined and adults of *Parasitohabditis*, *Micoletzya*, *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-7

Natural Occurrence of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) in the Aydin district of Turkey

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Poster / Nematodes. Wednesday, 16:30. NE-8

Use of entomopathogenic nematodes to control vine weevils on Chilean berry orchards

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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 Fillipjev 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 comparing 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 huaseri. These molecules could correspond to the genome of the first identified virus infecting an entomopathogenic nematode.

Poster / Nematodes. Wednesday, 16:30. NE-10
Cellular and humoral interactions between the white grub, Polyphilla adspersa Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes
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The interaction between the white grub larvae, Polyphilla adspersa Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes (EPN), Heterorhabditis bacteriophora and Steinernema glaseri was assessed. Differential Hemocyte Count (DHC) for the both second and third instar larvae of the white grub, granulocytes (65.25±%2.22) and plasmocytes (22.14±%1.14) were more 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 (IJ) of S. glaseri and H. bacteriophora into the insect hemocoe. The hemocoe 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 phenoloxidase 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 Polyphilla 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, entomopathogenic and parasite nematodes had a moderate frequency. The initial isolation of the collected nematodes carried out using morphometric data. Subsequently, molecular identification and phylogenetic analysis were performed using DNA sequences of ITS and 18S rDNA genes. The molecular data indicated that wg10 and wg19 isolates belong to Oscheius genus with 99% bootstrap support. Also, Nblast analysis introduced two isolates wg10 and wg19 as O. rugaolensis. The sequence of 185 gene O.
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% bootstrap 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 mellonella rather than healthy larvae. Further 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 small scale containers than in tissue culture container. The best storage temperatures for ScK were 13 and 20°C 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°C. 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, Bradyisia mabiusi (Diptera: Sciaridae): effect of dosage and application time*

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Fungus gnat, Bradyisia 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/cm² (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% humic acid 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 capturing 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/cm².

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 siricidica* 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. siricidica* 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. siricidica*, which is poorly understood and is a potential competitor of *D. siricidica* Kamona. *D. siricidica* 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 western Georgia. Insect damages mainly foliage trees and spread easily from forest vegetation to fruit orchards. The aim of the research was to study the efficacy of two species *S. carpocapsae* and local *S. thesami* against larvae of the gypsy moth in field conduction. Nematodes were reared produced in *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 m² 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 treat 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 we conducted with no prior EF exposure. Daily mortality was monitored for mycosis and placed on white traps in order to compare the total number of *S. feltiae* offspring produced. 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 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. 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. 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.

**Poster / Nematodes. Wednesday, 16:30. NE-16**

**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 fungi (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. 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. 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.

**Poster / Nematodes. Wednesday, 16:30. NE-19**

**Some observation on morphology and ecology of mollusc-parasitic nematode *Allionema appendiculatum***

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*Allionema appendiculatum* is a common larval parasite of many terrestrial molluscs. Its 3rd stage larvae (dauer juveniles) invade foot muscle of snails and slugs. Dauer juveniles
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 IJs 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 IJs. 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. An in 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.

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 recombiant protein by fusion expression with the partial polyhedrin in baculovirus.
A Colombian SiMNPV isolate (SiCOL-A) and its genotypic variant (SiCOL-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 SiCOL-A was determined and analyzed in the present study, consisting of 134,259 bp, encoding 144 putative open reading frames. Gene synteny maps showed great colinearity with genomes of other sequenced SiMNPVs. SiCOL-A genome displayed a ~1470 bp deletion localized within the main variable region among SiMNPV 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 SiMNPV 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 SiMNPV. Two bioinformatics approaches (relative similarity and bootscanning analysis) were used to explore the recombination hypothesis. Both analyses supported the hypothesis, showing a recombinant event involving the C terminus region of the chitinase ORF and the N terminus region of the gp37 ORF. This event resulted in the deletion of a genomic region including the Homologous Region 3 (HR3) and the 2I22 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 SiCOL-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.

Battleplateacinus, Photorimaeae opercula 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) is 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. opercula and T. absoluta) was determined by oral inoculation of each species 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 pif4, pif1, pif2, pif3, odv-e68, odv-e56 and pif6. The PCR products were cloned and sequenced. Comparative analysis of pif sequences of three isolates revealed high similarity with Photorimaeeae opercula granulovirus (PhopGV) previously reported in the Genbank. The topology of phylogenetic tree using concatenated deduced aminocacid 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.

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 also be used to develop a diagnostic tool.

Proteomic analysis of the occluded Tipula oleracea nodivirus (ToNV)
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 Panaeus 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 Betanuviridae clade representatives and distantly related to another Diptera-infecting nudivirus representative, the Drosophila innubria 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.

### 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 different prevalence. Understanding the controls of epizootics on BSW may provide valuable insight into possible biological controls for WM.

### 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 CASPS18/19 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.

### VI-8 Proteomic analysis and in vivo differential 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, subtractive 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 IV-6 expressing 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 IV-6. Were standardized optimal conditions for micro-projectile bombardment cotransfection of the vector DNA TOPO-295L-GFP-Cn10 and wild DNA from Iridovirus IV-6, being the ratio of 3:1 (vector: wild DNA) the more useful for obtained recombinant Iridovirus. Recombinant Iridovirus IV-6, was obtained by cotransfection of vector DNA TOPO-295L-GFP-Cn10 and wild DNA Iridovirus IV-6, using the technique of biologic 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 IV-6 and performing Western Blot tests to detect, for one hand, the insertion of both genes (GFP and Cn10) into the genome of IV-6 Iridovirus, and on the other hand, to verify 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 jujuba 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 MgCl₂, 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 multicosid 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 Alphabaculovirus. Preliminary bioassay data showed that the virus was active against either 2nd or 4th instars of jasmine moth. The calculated
We have developed a single baculovirus, named “Monobac”, for the production of recombinant Adeno-Associated Virus 8 vectors. The system was used for the production of rAAV8 using the Si9 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 enhancer gene, did not change viral potency. For example, a construct with E1 deleted/E2 zinc modified had the same potency as E1 deleted/E2 intact. These results suggest that the enhancers 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 capsid functions 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 to stabilize cypovirus capsids. However, we have observed 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 helixdestabilizing activity is RNA specific, lacks directionality, and could be inhibited by divalent ions, such as Mg²⁺, Mn²⁺, Ca²⁺, 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 RNA 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 Trichopilus 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, biolistic 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 biolistics was achieved with the AcNPV genome, when neonate Trichopilus 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 nucleus 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 LT₅₀ 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 results. 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 non-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 losses 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 Iliaviridae 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 virus 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 insect and 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 system. On the other hand, the BmDNV-2-derived promoter 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 Sf301 and larvae from *S. exigua* and *Trichoplusia ni*. Although we found higher 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 (CIV-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 straightforward 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 Heliotris 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 (CrlgEV) on FC populations from a range of geographic regions in South Africa**

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Consider the possibility of some geographic populations of the false codling moth, Thaumatomitia leucotreta (Meyrick) developing a reduced susceptibility to the baculovirus biopesticides, Cryptogram and Cryptex, as was the case with codling moth (CM), Cydia pomonella (L.) to the codling moth virus (CrPBV) in Germany, the search for new isolates of the T. leucotreta baculovirus (CrlgEV) 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 Cryptogram, 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 Cryptogram 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 basa lamina. Tracheal cell basa 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 alphanavirus 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 nucleopolyhedrovirus 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 epizootology 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 homologue genes 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 nucleopolyhedrovirus (SMINPV). 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 SI138null virus were ~15-fold less pathogenic to insects, which was attributed to a 100-fold reduction in OD infectious titer/OB. Inoculation of insects with SF138null OBs in mixtures with an optical brightener failed to restore the pathogenicity of SI138null 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 act150 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 ChcSNPV-TF1-A, -B, -C, -G and -H). The whole genome sequences of the
The cassava hornworm, Erinnyis ello ello (Lepidoptera: Sphingidae), is a very severe pest in cassava (Manihot esculenta) due to its worldwide 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 Betabaculavirus. 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 (PirGV). No typical homologous regions (hrs), cathepsin or chitinase genes were detected. Alphabetaculovirus 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.

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 were 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 st29 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.

Analysis of genetic interactions among four non-essential genes of BmNPV
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Nucleopolyhedroviruses (NPV) produce copious amounts of polyhedrin (Poh) 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 Poh 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.

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 cubeshaped 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 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-33-STU
Poster / Viruses. Wednesday, 16:30. VI-34 STU
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 on the sucrose content in sugarcane, characteristically seriously affected by the presence of the stem borers complex, difficulty 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 a soil tank until death. A total of 445 larvae were observed infected by SfMNPV OBs in a model laboratory system. A soil incorporation bioassay technique was calibrated for granulovirus investigation. For granulin produced in hen eggs. Two samples from Boyacá and one from Santander were positive by the three immunologic 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 powerful for detecting granulovirus in field samples and four detected virus will be amplified in the insect for a further characterization and biopesticide development.

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 (5x10^3 - 5x10^6 OBs/ml). The LC50 value was estimated at 2.3 x 10^3 OBs/ml. The gut pH of E. fetida was estimated to be pH 5.0-6.0 using pH indicators. Earthworms transported 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.

Effects of rearing temperature on the susceptibility of larvae of the smaller tea tortrix, Adoxophyes honmaei (Lepidoptera: Tortricidae) to A. honmaei 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 entomopathogens in the host. The smaller tea tortrix, Adoxophyes honmaei, 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. honmaei 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. honmaei larvae to A. honmaei nucleopolyhedrovirus (AdhoNPV). Fifth instar larvae of A. honmaei 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. honmaei larvae was reduced with an increase in rearing temperature. No AdhoNPV-infected larvae were observed when larvae were reared at 34°C. The infection rate of A. honmaei 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.

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 replicate 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, confounding both 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 WhNV and FHV protein A, and represents an important advance toward understanding nodaviral RNA replication.
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 of 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
Brian Federici
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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 cm². 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.
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 conclusions 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. Inverteb.) Thursday, 8:00-10:00**

**Emerging Tools for Aquatic Pathogen Discovery and Description**

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 in 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.

**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 its 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.

**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
Environmental DNA as a tool for detection and identification of aquatic parasites: known unknowns and just plain unknowns

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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 just plain unknowns. Initially, four pathogens of interest to aquatic hosts. eDNA analyses should therefore be considered as a ‘tool in between presence within the matrix and outcomes in hosts. eDNA analyses should therefore be considered as a ‘tool in between presence within the matrix and outcomes in hosts.

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 *Macrophagos pygmaeus* are suggested as effective components within a new control strategy against the insect in 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.

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 *B. longissima* and *C. variegate* pests are unknown. Initially, four isolates of *H. bacteriophora*, UV resistant *H. bacteriophora* Shandong (HbSD), *B. longissima* and *C. variegate* were bioasayed 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 (JJs) for six days, mortality was correlated with dosage, and the LC50 was at 9.35 JJs for *B. longissima* and *C. variegate* as compared with that at 8.56 JJs 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
Problems associated with the use of chemical pesticides include the development of resistance by pests and adverse effects on non-target species and the environment. As an alternative, entomopathogenic nematodes (EPNs) have been studied as potential biological control agents for various insect pests. 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, ALG03, ALG15, ALG16 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 made it possible 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. y irgalemense 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 feeding method from soil collected

Contributed paper. Thursday, 8:30. 209

Propects for using Entomopathogenic Nematodes to Control the Vine Mealbug, Planococcus ficus, in South African Vineyards

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Planococcus ficus (Signoret), the vine mealybug, is regarded as a major pest insect of the South African grape industry. Mealbugs 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 mainly occur mostly on the aerial part of plants, but also on the roots. Both local Heterorhabditis 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 coding 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). The 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, ALG03, ALG15, ALG16 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 made it possible 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.
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 chelorrhabidion 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 peloderaurus, 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 Raif-Udo Ehlers
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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 culture. The methodology to produce stable inbred lines for steinernematids needs further investigation, as these nematodes are amphibimetic 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 Mariam Chubinishvili, Tsitsia Chkhubianishvili, Manana Kakhadze, Iatamze Malania Kanchaveli Institute of Plant Protection, Agricultural University of Georgia, 240, David Agmashenebeli Alley, 0159 Tbilisi, 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 agroecosystems 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 paper. Thursday, 8:00. 215
Interactions between salivary gland hypertrophy virus and tsetse microbiota Gülber Demirbas Uzel1, Vangelis Doudoumis2, Antonios Augustinos3, Gisele Ouedroogo1, Andrew Parker1, Drion Boucias2, Kostas Bourtzis2, Adly Abd-Alla3,1
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Many species of tsetse files 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 gossinidia, 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 1) 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 files (mainly G. morsitans morsitans and G. austeni) clearly indicated a negative interaction between SGHV and Wolbachia. The results of this study have been presented at the 7th International Conference of the Société de Lutte Biologique against Insects (Puebla, Mexico, 2019) and in a manuscript under review. Further research is needed to identify the role of the SGHV in the transgeneration transmission of Wolbachia-infected Glossina species.
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.

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.

Invertebrate iridescent virus 6 (IV-6) is a nucleocytoplasmic virus with a 212 kb-long double-stranded DNA genome that encodes 215 putative open reading frames. The IV-6 virus 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 IV-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 IV-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 (infecotome). 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.

Ascorviruses (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 ascorviruses 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. littura 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.

Insect metamorphosis is predominantly regulated by two hormones, juvenile hormone (JH) and ecdysone. During the final instar, a dramatic decrease in JH titers is required for the induction of pupation. JH is metabolized by two enzymes, JH
nucleopolyhedrovirus (NPV), 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 >LC50 (1.0 x 10^6 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 endocronological 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 Δptp-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 serotonine 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, 9:45. 222
The essential baculovirus protein VP1054 is a hijacked cellular PRUs.
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 purine-rich 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
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 counteract. 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.

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.
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 micro-parasite 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. Important, 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, 8:45 226

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, 9:00 227

Experimental evolution in silico: host-parasite coevolution versus parasite adaptation
Jakob Strauß\textsuperscript{1}, Philip Crain\textsuperscript{2}, Sultan Beshir\textsuperscript{1}, Joachim Kurtz\textsuperscript{1}, Hinrich Schulenburg\textsuperscript{2}, Arndt Telschow\textsuperscript{1}
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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, 9:30 229

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
Jacqueline Hollenstein 1, Joachim Kurtz 2, Hinrich Schulenburg 3, Heiko Liesegang 1
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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 paracrysal 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 PilCR quorum sensor allow the bacteria to damage the intestinal barrier and to gain access to the haemocoel. After the death of the insect, PilCR activates transcription of a gene encoding a second quorum sensor, NpR. NpR 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. PilCR, NpR and Rap are quorum sensing regulators belonging to the RNPP family. Their activity depends on the signalling peptides PapR, NpX 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 PilR-PapR complex induces the production of virulence factors allowing the bacteria to kill the insect. NpR-NpX 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
Elke Genersch; Anne Fürnhaus; Eva Garcia-Gonzalez; Gillian Hertlein; Lena Poppinga
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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.
**Contribution 234**

**Title**: Vibrio and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes  
**Authors**: Audrey Vanhove, Annick Jacq, Frédérique Le Roux, Tristan Rubio, Alexandra Calteau, Evelyne Bachère, Julie Nicod, Agnès Vergnes, Astrid Lemire, Guillaume Charrister, and Delphine Destoumieux-Garzón  
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**Abstract**: 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 microbial 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 cytolytic 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 genes involved in antioxidant defenses 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.

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**Contribution 235**

**Title**: Establishing the fungal entomopathogen Beauveria bassiana (Ascomycota: Hypocreales) as an endophyte in cucurbits for managing Zucchini Yellow Mosaic Virus (ZYMV)  
**Authors**: Lara R. Jaber and Nidal Salem  
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**Abstract**: 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.
Colonized plants with entomopathogenic fungi produce mortality in Spodoptera littoralis (Boisdual) (Lepidoptera: Noctuidae) larvae
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This study aimed to evaluate the ability of entomopathogenic fungi to colonize endophytically plants for Spodoptera littoralis (Boisdual) (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 tobacco (Ncypersicon esculentum Mill), melon (Cucumis melo L., hybrid F1- Galaia) 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 EBBb 01/33-Su demonstrated an increasing percentage of colonization of B. bassiana 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.
increased attention and vigilance, 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_ Hübnr 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 (92.5%) 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 6th 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 budding 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 RT-PCR. 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

Hacer Muratoglu 1, Remziye Naalacioglu 2, Basil Arif 1, Zihni Demirbag 2, 1 Karadeniz Technical University, Faculty of Sciences, Department of Molecular Biology and Genetic, 61080 Trabzon, Turkey; 2 Karadeniz Technical University, Faculty of Sciences, Department of Biology, 61080 Trabzon, Turkey; 3 Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada
Address for Correspondence: zihni@ktu.edu.tr

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

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 430071, P.R. China
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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 electron-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.coil, 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.
The leucines in the transmembrane domain of Autographa californica nucleopolyhedrovirus Ac76 are important for intranuclear microvesicle formation

Denghui Wei, Yan Wang, Xiaohe Zhang, Meijin Yuan, Kai Yang, State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

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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 L26, L29 and L33 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.

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, 105 Xingang Road West, Guangzhou 510260, China.

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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.

Contributed paper. Thursday, 15:30.
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Seit 2008 werden sie unter neuem Namen weitergeführt:
„Berichte aus dem Julius Kühn-Institut“

Heft 151, 2010: NEPTUN 2009 – Weinbau. Dietmar Roßberg, 19 S.
Heft 162, 2011: Viertes Nachwuchswissenschaftlerforum 2011 - Abstracts -. , 62 S.
Heft 168, 2013: Untersuchungen zur Bildung von Furocumarinen in Knollensellerie in Abhängigkeit von Pathogenbefall und Pflanzenschutz. Andy Hintenaus, 92 S.
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<td>Bacteria P5</td>
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<td>Microsporidia P4</td>
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<td>Fungi P2</td>
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<td>Viruses P3</td>
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<tr>
<td>7:30-16:30</td>
<td>Registration</td>
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<tr>
<td>8:00-10:00</td>
<td>Symposium 7 (Diseases of Beneficial Invertebrates)</td>
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<tr>
<td></td>
<td>Emerging Tools for Aquatic Pathogen Discovery and Description</td>
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<td>Early mortality syndrome is an infectious disease with a bacterial</td>
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<td>Policy, phylogeny, and the parasite</td>
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<td></td>
<td>Grant D. Sentistford</td>
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<td>The Next Generation of Crustacean Health: Disease</td>
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<td>Diagnostics Using Modern Transcriptomics K. Fraser Clark</td>
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<td>Environmental DNA as a tool for detection and identification of</td>
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<td>aquatic parasites: known unknowns and just plain unknowns</td>
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<td>Hanna Harritkainen</td>
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<td>8:00-10:00</td>
<td>Symposium (DFG Priority Program) P5</td>
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<td>Organiser: Joachim Kurtz</td>
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<td>Host Parasite Coevolution</td>
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<td>14:00-16:00</td>
<td>Symposium 8 (Cross-Divisional) Host-Pathogen Ecology at the Molecular</td>
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<td>Level: Gene Regulation and Environment Sensing</td>
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<td>The Bacillus thuringiensis way of life: communicate to kill and</td>
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<td>survive in the insect host Didier Lereclus</td>
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<td>The interplay of Paenibacillus larvae with honey larvae during</td>
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<td>infection Elke Genersch</td>
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<td>Antimicrobial defense and persistent infection in insects revisited</td>
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<td>Jens Rolf</td>
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<td>Vibrio and the intraphagosomal environment: how an oyster pathogen</td>
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<td>evades intracellular killing in oyster hemocytes Delphine</td>
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<td>14:00-16:00</td>
<td>Contributed Papers</td>
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<td>Microbial Control 4</td>
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<td>Viruses 7</td>
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<td>16:00-16:30</td>
<td>Student Business Meeting P4</td>
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<td>18:30 Departure from Hotels to Banquet</td>
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<td>19:00-1:00</td>
<td>Reception and Banquet Alte Lokhalle</td>
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</table>

### Seeing you in Vancouver for SIP 2015!