42nd Annual Meeting of the Society for Invertebrate Pathology

Park City, Utah

August 16–20, 2009

All meetings and all meals will be held at the Grand Summit Hotel. Your conference badge is your entrance and meal ticket, please keep it with you.

SUNDAY — 16 August

8:00 – 17:00	SIP Executive Council Meeting	*Suite #620*
15:00 – 18:00	Registration Open	*Mezzanine*
18:00 – 21:00	Mixer / Welcome Reception	*Ballroom*

MONDAY — 17 August

6:30 – 8:30	Breakfast Buffet	*Outdoor Pavillion*
7:00 – 17:00	Registration Open	*Kokopelli Lobby*
8:30 – 10:00	**Opening Ceremony** **Award Presentations** **Founder's Lecture**	*Ballroom*
10:00 – 10:30	Break	*Kokopelli Lobby*
10:30 – 12:30	**Symposia:** *Insect RNA Viruses: Advances and Applications *Diseases in Populations of Beneficial Invertebrates	*Ballroom II* *Painted Horse*
10:30 – 12:30	**Contributed Papers:** *Bacteria I	*White Pine*
12:30 – 14:00	Lunch Buffet	*Outdoor Pavillion*
12:30 – 14:00	JIP Editorial Board Meeting & Lunch	*Suite #620*
14:00 – 16:00	**Symposium:** *Epizootiology and Its Impact on Microbial Control: Honoring the Work of Jim Fuxa	*Ballroom II*
14:00 – 16:00	**Contributed Papers:** *Invertebrate Immunity *Nematodes I	*White Pine* *Painted Horse*
16:00 – 16:30	Break	*Kokopelli Lobby*
16:30 – 17:30	**POSTER 1 -** Bacteria, Microbial Control, Nematodes	*Kokopelli I*
17:30 – 19:00	Dinner Buffet	*Outdoor Pavillion*
19:00 – 20:00	**Division Business Meetings** *Bacteria Division *Fungus Division *Microsporidia Division *Nematode Division *Virus Division	*Ballroom III* *White Pine I* *Painted Horse* *White Pine II* *Ballroom II*
20:00 – 21:00	**Microsporidia Division Workshop** *Staining Techniques Used for Microsporidia Infecting Invertebrates	*Painted Horse*
20:00 – 21:00	**Virus Division Workshop** *Advances in Invertebrate Cell Culture	*Ballroom II*

TUESDAY – 18 August

6:30 – 8:00	Breakfast Buffet	*Outdoor Pavillion*
7:00 – 12:30	Registration Open	*Kokopelli Lobby*
8:00 – 10:00	**Plenary Symposium** The Host-Pathogen Dance: Interactions Between Insect Hosts and Their Pathogens	*Ballroom*
10:00 – 10:30	Break	*Kokopelli Lobby*
10:30 – 12:30	**Symposia:** **Bt* Resistance in the Real World *Fungi in Soil Habitats— Doing it in the Dirt	*Ballroom II* *Painted Horse*
10:30 – 12:30	**Contributed Papers:** *Virus I	*White Pine*
12:30 – 16:00	**EXCURSION** Utah Olympic Park. Pre-registration required. Box Lunch included.	*Board bus from hotel lobby*
17:00 – 18:30	**5K Fun Run/Walk** Pre-registration required.	*Ride gondola to Red Pine Lodge*
19:00 – 23:00	**BBQ Cookout**	*Ride gondola to Red Pine Lodge*
23:00	Optional: Meet in *The Cabin* for drinks	*The Cabin Restaurant*

Wednesday — 19 August

6:30 – 8:00	Breakfast Buffet	*Outdoor Pavillion*
7:00 – 12:00	Registration Open	*Kokopelli Lobby*
8:00 – 10:00	**Symposium:** *Invertebrate Antiviral Response	*Ballroom II*
8:00 – 10:00	**Contributed Papers:** *Bacteria II *Fungi I	*White Pine* *Painted Horse*
10:00 – 10:30	Break	*Kokopelli Lobby*
10:30 – 12:30	**Symposium:** **Bt* the Bacterium, Ecology and Infection	*Ballroom II*

Agenda continued on back inside cover

IMPORTANT NOTE ABOUT POSTERS

Posters should be displayed by 8:00 Monday or Wednesday in Kokopelli I. Posters must be removed no later than 20:00 on Tuesday and 15:30 on Thursday. Presenters should stand by their posters during the appropriate poster session.

MEALS

Meals are paid for in advance, and are included in the registration fee. You will need to show your conference badge to restaurant staff.

Cover Photograph: Overview of Deer Valley, Utah near Park City

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	Zhihong (Rose) Hu	Wuhan Institute of Virology, Chinese Academy of Sciences Xiao Hong Shan 44, Wuhan, Hubei, 430071, P. R. OF CHINA **Phone**: +86 27 87197180 **Email:** huzh@wh.iov.cn
	Jeffrey Lord	GMPRC, USDA-ARS 1515 College Ave, Manhattan, KS 66502, USA **Phone**: (785) 776-2705 **FAX**: (785) 537-5584 **Email:** jeff.lord@ars.usda.gov
	Christina Nielsen-Le Roux	INRA, Unité Génétique microbienne et Environnement, INRA La Minière, Guyancourt, 78285, FRANCE **Phone**: +33 1 30 83 36 42 **FAX**: +33 1 30 43 80 97 **Email:** christina.nielsen@jouy.inra.fr
Executive Secretary	**Cecilia Schmitt**	Society for Invertebrate Pathology PO Box 11, Marceline, MO 64658 USA **Phone**: Toll Free in North America: 888-486-1505 Outside of North America: 660-376-3586 **Email**: sip@sipweb.org

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2009 PROGRAM

NOTE

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

-STU Indicates papers being judged for GRADUATE STUDENT presentation awards

100 Indicates abstract number for ORAL presentations

B-10 Indicates abstract number for POSTER presentations

SUNDAY — 16 August

08:00 - 17:00 **SIP Council Meeting**
Grand Summit Suite #620 (sixth floor)

15:00 - 18:00 **Registration Open**
Grand Summit - Mezzanine

18:00 - 21:00 **Mixer**
Kokopelli Ballroom

MONDAY — 17 August

06:30 - 08:30 **BREAKFAST BUFFET**
Outdoor Pavillion - behind the hotel

07:00 - 17:00 **Registration Open**
Kokopelli Lobby

08:30 - 10:00 **Opening Ceremonies, Award Presentations, & SIP Founders' Lecture**
Kokopelli Ballroom II-III

Opening Ceremonies
Rosalind R. James and Donald W. Roberts, Chairs, Organizing Committee
Mark S. Goettel, President, SIP

Award Presentations
Mauro-Martignoni Award Presentation
Chris Lomer Award Presentation
Announcement of Division Travel Awards

Founders' Lecture
James J. Becnel, Chair, Founders' Lecture Committee

Honoree: Donald W. Roberts
Lecturer: Raymond J. St. Leger

"50 Years of Leadership in Insect Pathology"

10:00 - 10:30 **BREAK** *Kokopelli Lobby*

Symposium (Virus Division) Monday, 10:30 - 12:30
Kokopelli Ballroom II

Insect RNA Viruses: Advances and Applications

Organizers/Moderators: Bryony Bonning, Karyn Johnson

10:30 **1** **The *Tetraviridae*: Prospects for targeted drug delivery.** *Rosemary Dorrington* - Rhodes University, ***Marli Vlok*** - Rhodes University, ***Michele Tomasicchio*** - Rhodes University

11:00 **2** **Assembly of multi-layered viral nanoparticles: A new approach for vaccine design.** *Anette Schneemann* - The Scripps Research Institute

11:30 **3** **Alphavirus transducing systems.** *Ken Olson* - Colorado State University

12:00 **4** **The dicistroviruse: Advances and applications.** *Bryony Bonning* - Iowa State University

Symposium (Beneficials Division) Monday, 10:30 - 12:30
Painted Horse I-II

Diseases in Populations of Beneficial Invertebrates

Organizer/Moderator: Grant Stentiford

10:30 **5** **Crustacean diseases in European legislation: An overview of recent developments.** *Grant Stentiford* - European Community Reference Laboratory for Crustacean Diseases

11:00 **6** **Social insects and their parasites in the wild – What do we know and where should we go?** *Mark Brown* - Royal Holloway, University of London

11:30 **7** **Fungal pathogens from the genus *Ascosphaera* in populations of honey bees and solitary bees.** *Jørgen Eilenberg* - University of Copenhagen, ***Annette B. Jensen*** - University of Copenhagen, ***Rosalind R. James*** - USDA-ARS Pollinating Insects Research Unit, ***Bo V. Pedersen*** - University of Copenhagen, ***Svetlana Vojvodic*** - University of Copenhagen, ***Anja A. Wynns*** - University of Copenhagen

12:00 **8** **Disease transmission in honey bees.** *Elke Genersch* - Institute for Bee Research

12:15 **9-STU** **Disease profile of adult and juvenile edible crab (Cancer pagurus) populations from the English Channel Fishery, UK.** *Kelly Bateman* - Cefas, ***Stentiford Grant*** - Cefas

CONTRIBUTED PAPERS Monday, 10:30 - 12:30
White Pine I-II

Bacteria I

Moderator: Hyun-Woo Park

10:30 **10** **Proteomic analysis of the crystal and spore mixture from six *Bt* strains to search for novel mosquitocidal proteins.** *Yunjun Sun* - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University, ***Hyun-Woo Park*** - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University

10:45 **11-STU** **Comparing the midgut epithelial regenerative response in *Bt*- susceptible and -resistant *Heliothis virescens* larvae.** *Anaïs Castagnola* - University of Tennessee, Knoxville, ***Juan Luis Jurat-Fuentes*** - University of Tennesee, Knoxville, ***Shigetoshi Eda*** - University of Tennesee, Knoxville

12 *moved to 92*

11:00 **13-STU** **Lpss are virulence factors of the Mexican *Serratia entomophila mor4.1* to *Phyllophaga blanchardi* larvae (Coleoptera).** *Zitlhally Rodriguez-Segura* - Centro de Investigación en Biotecnología,2 Universidad Autónoma del Estado de Morelos, ***Jean Chen*** - Depts. of Cell Biology and Neuroscience, ***Francisco Villalobos*** - Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, ***Sarjeet Gill*** - Depts. of Cell Biology and Neuroscience, Maria Eugenia ***Nuñez-Valdez*** - Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos

11:15 **14-STU** **Overproduction of Cry2ac by modification of nucleotide sequences between *rbs* and start codon in.** *Faiza Saleem* - School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan, ***Hyun-Woo Park*** - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center Florida A & M University, Panama City, Florida 32405, U.S.A., ***Muhammad Akhtar*** - School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan, ***Abdul Rauf Shakoori*** - School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan

11:30 **15** **High expression of pathogen-induced genes in a *Spodoptera exigua* colony resistant to *Bacillus thuringiensis*.** Patricia ***Hernández-Martínez*** - Department of Genetics, Universitat de València, Spain, ***Gloria Navarro-Cerrillo*** - Department of Genetics, Universitat de València, Spain, ***Ruud A. de Maagd*** - Plant Research International B. V., The Netherlands, ***William Moar*** - Department of Entomology and Plant Pathology, Auburn University, USA, ***Juan Ferré*** - Department of Genetics, Universitat de València, Spain, ***Baltasar Escriche*** - Department of Genetics, Universitat de València, Spain, *Salvador Herrero* - Department of Genetics, Universitat de València, Spain

11:45 **16** **Cry1ac receptors and susceptibility during heliothine larval development.** ***Weibing Shi*** - Zhejiang University, ***Cris Oppert*** - University of Tennessee, *Juan Luis Jurat-Fuentes* - University of Tennessee

12:00 **17** **The development of the *Cry8*-type genes from *Bacillus thuringiensis* toxic to scarabs.** ***Guixin Yan*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Fuping Song*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Zhihong Lang*** - Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, ***Dafang Huang*** - Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, *Jie Zhang* - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences

12:30 - 14:00 **LUNCH BUFFET** *Oudoor Pavillion*

12:30 - 14:00 **JIP Editorial Board Meeting**
Grand Summit Suite #620 (sixth floor)

Symposium (Cross Divisional) Monday, 14:00 - 16:10
Kokopelli Ballroom II

Epizootiology and Its Impact on Microbial Control—Honoring the Work of Jim Fuxa

Moderator: David Shapiro-Ilan
Organizers: David Shapiro-Ilan, Ann Hajek

14:00 **18** **Epizootiology of fungi.** *Ann E. Hajek* - Department of Entomology

14:30 **19** **Epizootiology of nematodes.** *Harry K. Kaya* - University of California, Department of Nematology

15:00 **20** **Epizootiology of microsporidia.** *David Oi* - USDA-ARS Center for Medical, Agricultural, & Veterinary Entomology

15:30 **21** **Nucleopolyhedrovirus epizootiology: One researcher's perspective.** *James Fuxa* - Retired Professor

16:00 **22** **Panel Discussion, Summary.** *David Shapiro-Ilan* - USDA-ARS

CONTRIBUTED PAPERS Monday, 14:00 - 16:00

White Pine I-II

Invertebrate Immunity

14:00 **23-STU** **Consumption of *Bacillus thuringiensis* and selection for *Bt*resistance is associated with changes in insect immune function.** *Jerry Ericsson* - Simon Fraser University, ***Judith Myers*** - University of British Columbia, ***Carl Lowenberger*** - Simon Fraser University

14:15 **24-STU** **Upregulation of circulating hemocytes in response to bacterial challenge is mediated by biogenic amines via *rac1* signal.** *Geun Seob Kim* - Andong National University, ***Yonggyun Kim*** - Andong National University

25 *withdrawn*

14:30 **26** **Transcriptome analysis of pathogen exposed *Spodoptera exigua* larvae.** *Salvador Herrero* - Department of Genetics. Universitat de Valencia, ***Heiko Vogel*** - Department of Entomology, Max Planck Institute for Chemical Ecology

14:45 **27** **Detection and analysis of innate immune genes of the termite.** *Drion Boucias* - University of Florida, ***John Denton*** - University of Florida, ***Tamer Salem*** - University of Florida, ***Michael Scharf*** - University of Florida

MONDAY PM

CONTRIBUTED PAPERS — Monday, 14:00 - 16:00
Painted Horse I-II

Nematodes I

Moderator: Kelly Sims

14:00 **28** **The screening symbiotic bacteria and its purification and gene clone of insecticidal proteins from *Xenorhabdus poinarii SY5*.** *Bin Cong* - Laboratory for Bio-control,Shenyang Agricultural University, ***Huan Wang*** - Laboratory for Bio-control, Shenyang Agricultural University, ***Xihua Wang*** - Laboratory for Bio-control, Shenyang Agricultural University, ***Hui Dong*** - Laboratory for Bio-control, Shenyang Agricultural University, ***Haitao Qian*** - Laboratory for Bio-control, Shenyang Agricultural University

14:15 **29** **The *Wolbachia* endosymbiont as a potential anti-filarial nematode target.** ***Bo Wu*** - Molecular Parasitology Division, ***Jacopo Novelli*** - Molecular Parasitology Division, ***Jeremy Foster*** - Molecular Parasitology Division, *Barton Slatko* - Molecular Parasitology Division

14:30 **30-STU** **Mutational analysis yields insight into the function of *nilb*, a specificity determinant in an animal-bacteria symbiosis.** *John Chaston* - Department of Bacteriology, University of Wisconsin-Madison, ***Heidi Goodrich-Blair*** - Department of Bacteriology, University of Wisconsin-Madison

14:45 **31-STU** **Transcriptional profiling of trait deterioration in the insect pathogenic nematode *Heterorhabditis bacteriophora*.** *Bishwo Adhikari* - Department of Biology, Brigham Young University, ***Chin-Yo Lin*** - Department of Microbiology and Molecular Biology, Brigham Young University, ***Parwinder Grewal*** - Department of Entomology, The Oho State University-OARDC, ***David Shapiro-Ilan*** - USDA-ARS, Southeastern Fruit and Nut Research Laboratory, ***Byron Adams*** - Department of Biology, Brigham Young University

15:00 **32** **Genetic breeding for heat tolerance of *Heterorhabitis bacteriophora*. *John Mukuka*** - Kiel University, ***Olaf Strauch*** - Kiel University, *Ralf Udo Ehlers* - Kiel University

15:15 **33-STU** **A model system for the identification of 'insects parasitism genes' and the investigation of 'parasitism gene' evolution.** *Scott Peat* - Department of Biology, Brigham Young University, ***Byron Adams*** - Department of Biology, Brigham Young University

15:30 **34-STU** **Evaluating the cost of environmental RNAi resistance in *Caenorhabditis elegans*.** *Stephen Jenkins* - Department of Biology, Brigham Young University, ***Barry Pittendrigh*** - Department of Entomology, University of Illinois at Urbana-Champaign, ***David Onstad*** - Department of Crop Sciences, University of Illinois at Urbana-Champaign, ***Byron Adams*** - Department of Biology, Brigham Young University

16:00 - 16:30 **BREAK** *Kokopelli Lobby*

Monday, 16:30 - 17:30
Kokopelli Ballroom I

POSTERS 1

Posters will be displayed from Monday 8:00 to Tuesday 17:30.

BACTERIA

B1-STU ***Manduca sexta* aminopetidase n and alkaline phosphatase are involved in binding with the oligomeric structure of Cry1ab toxin.** *Ivan Arenas* - Instituo de biotecnología, UNAM., ***Alejandra Bravo*** - Instituto de bitecnología, UNAM., ***Mario Soberón*** - Instituto de biotecnología UNAM, ***Isabel Gómez*** - Instituto de biotecnología, UNAM.

B-2 **The effects of *Bacillus thuringiensis* on adult emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae).** *Leah S. Bauer* - USDA Forest Service, Northern Research Station, ***Diana Londono*** - Michigan State University, ***Libs John*** - Pyllom LLC

B-3 **Characterization of Cry1ac binding and pore formation in Cry1ac-resistant *Helicoverpa zea* (Boddie).** *Silvia Caccia* - University of Valencia, ***Jayadevi Chandrashekhar*** - Auburn University, ***William J Moar*** - Auburn University, ***Juan Ferré*** - University of Valencia

B-4-STU **Silencing of *Manduca sexta* aminopeptidase n1 by feeding double stranded RNA to study the receptor function *in vivo*.** *Biviana Flores-Escobar* - Instituto de Biotecnología UNAM, ***Alejandra Bravo*** - Instituto de Biotecnología UNAM, ***Mario Soberón*** - Instituto de Biotecnología UNAM, ***Isabel Gómez*** - Instituto de Biotecnología UNAM

B-5 **Loop residues of the receptor binding domain of *Bacillus thuringiensis* Cry11ba toxin are important for mosquitocidal activity.** *Supaporn Likitvivatanavong* - UCR, ***Karlygash Aimanova*** - UCR, ***Sarjeet Gill*** - UCR

MONDAY PM

B-6-STU **Cloning of *Bacillus thuringiensis* plasmids using a modified plasmid capture system.** *Qin Liu* - Department of Agricultural Biotechnology, Seoul National University, ***Jong Yul Roh*** - Department of Agricultural Biotechnology, Seoul National University, ***Yong Wang*** - Department of Agricultural Biotechnology, Seoul National University, ***Jae Young Choi*** - Research Institute for Agriculture and Life Sciences, Seoul National University, ***Hee Jin Shim*** - Department of Agricultural Biotechnology, Seoul National University, ***Hong Guang Xu*** - Department of Agricultural Biotechnology, Seoul National University, ***Xueying Tao*** - Department of Agricultural Biotechnology, Seoul National University, ***Byung Rae Jin*** - Department of Agricultural Biotechnology, Seoul National University, ***Yeon Ho Je*** - Department of Agricultural Biotechnology, Seoul National University

B-7 **Effects of a new member of the toxin complex family on insects.** *Sean Marshall* - AgResearch, ***Sandra Jones*** - AgResearch, ***Mark Hurst*** - AgResearch

B-8 **Discovery of novel pesticidal protein genes in *Bacillus thuringiensis* using *de novo* sequencing.** *Kimberly Sampson* - Athenix Corp., ***Ethan Dunn*** - Athenix Corp., ***Jessica Zeigler*** - Athenix Corp., ***Daniel Tomso*** - Athenix Corp.

B-9 **Screening and cloning of a *vip3A* gene from a *Bacillus thuringiensis* strain toxic to lepidopteran pests.** *Joseilde O. Silva-Werneck* - Embrapa Genetic Resources and Biotechnology, ***Elias F. Sabiá Júnior*** - University of Brasilia, ***Laíse S. Evangelista*** - University of Brasilia, ***Thatianny A.L. Silva*** - University of Brasilia, ***Vitor R. Valdez*** - University of Brasilia, ***Bergmann M. Ribeiro*** - University of Brasilia, ***Rose G. Monnerat*** - Embrapa Genetic Resources and Biotechnology

B-10-STU **The Aealp is an important receptor molecule against Cry1AA.** *Takaya Tomokiyo* - Graduate School of Agriculture, Hokkaido University, ***Hisanori Bando*** - Graduate School of Agriculture, Hokkaido University, ***Shin-ichiro Asano*** - Graduate School of Agriculture, Hokkaido University

B-11 **Virulence causing ability of *Serratia proteamaculans* strains towards the grass grub *Costeyltra zealandica*.** *Mark Hurst* - Agresearch, ***Maureen O'Callaghan*** - Agresaerch, ***Joanne Calder*** - Agresearch, ***Lincoln Harper*** - Agresearch

B-12-STU **Insecticidal activity and mode of action of Cry8d against Japanese beetle.** *Takuya Yamaguchi* - Hokkaido Univ., ***Ken Sahara*** - Hokkaido Univ., ***Hisanori Bando*** - Hokkaido Univ., ***Shin-ichiro Asano*** - Hokkaido Univ.

B-13 **Expression of a Cry1ac-gfp fusion protein in *Bacillus thuringiensis*.** *Hui Yang* - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Fuping Song*** - Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Jie Zhang*** - Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Dafang Huang*** - Biotechnology Research Institute, Chinese Academy of Agricultural Sciences

B-14 **Research on *Bacillus thuringiensis* against animal and plant parasitic nemoatodes.** *Ziniu Yu* - State Key Laboratory of Agricultural Microbiology, National Enginerring Research Center for Microbial Pesticides, ***Jianhong Li*** - State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, ***Mingshun Li*** - State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, ***Ming Sun*** - State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides

B-15 **Characteristics of a *B. thuringiensis spoIIID* gene mutant.** ***Qianqian Zhang*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Junlan Ma*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Fuping Song*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, *Jie Zhang* - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Dafang Huang*** - Biotechnology Research Institute, Chinese Academy of Agricultural Sciences

B-16 **Transcriptomic and proteomic analysis of Bt-resistant *Spodoptera frugiperda* sf9 cell lines.** *Claude Castella* - INRA, ***Pierre Barbero*** - INRA, ***Trang Tran*** - INRA

MONDAY PM

B-17 **Cross-resistance of Cry1ac-selected Asian corn borer to other Bt toxins.** ***Hailiang Han*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Kanglai He*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Guangtao Li*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Zhenying Wang*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Jie Zhang*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences

B-18 **Characterization and organization of novel *cry* genes isolated from mosquitocidal *Bacillus thuringiensis* strain.** ***Yu Karatani*** - Kinki University, ***Akiyoshi Kasugai*** - Kinki University, ***So Takebe*** - Kinki University

B-19 ***Candidatus liberibacter asiaticus* propagation in psyllid cell cultures.** Mizuri ***Marutani-Hert*** - USDA-ARS, ***Wayne Hunter*** - USDA-ARS, ***David Hall*** - USDA-ARS

B-20 **Metagenomic approach to psyllid microbes.** ***Mizuri Marutani-Hert*** - USDA-ARS, ***Wayne Hunter*** - USDA-ARS, ***David Hall*** - USDA-ARS

B-21-STU **Characterisitics of a *Bacillus thuringiensis* strain fcd114.** ***Xiaodong Sun*** - State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Changlong Shu*** - State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***JiGuo Gao*** - Northeast Agricultural University, ***Fuping Song*** - State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, ***Jie Zhang*** - State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences

B-22 *withdrawn*

B-23 **Toxicity of *Bacillus thuringiensis* and its combined effect with *Habrobracon hebetor* to *Plodia interpunctella*.** Akinkurolere ***Rotimi Oluwafemi*** - Huazhong Agricultural University, Wuhan, China, ***Hong-Yu Zhang*** - Huazhong Agricultural University, Wuhan, China

MICROBIAL CONTROL

MC-1 **A new microbial insecticide from *Chromobacterium subtsugae*.** ***Lisa Chanbusarakum*** - Marrone Organic Innovations, ***Eunice Tan*** - Marrone Organic Innovations, ***Ratnakar Asolkar*** - Marrone Organic Innovations, ***Huazang Huang*** - Marrone Organic Innovations, ***Pam Marrone*** - Marrone Organic Innovations

MC-2 **Lethal encounters in *Synanthedon* tunnels: apple clearwing moth larvae and entompthogenic fungi and nematodes.** ***Joan Cossentine*** - AAFC, ***Paul Randall*** - AAFC, ***John Bissett*** - AAFC, ***Lerry Lacey*** - USDA-ARS

MC-3 **Formulation and evaluation of aquatic mosquito larvicides based on *Bacillus thuringiensis subsp. israelensis* in Vietnam.** ***Binh Dinh Ngo*** - Institute of Biotechnology, ***Tuan Dinh Nguyen*** - Institute of Biotechnology, ***Ha Thu Trinh*** - Institute of Biotechnology, ***Thanh Quang Le*** - Ministry of Science and Technology

MC-4 **Shelf-life of *Beauveria bassiana* conidia in gaseous atmospheres under high temperature regimes.** ***Marcos Faria*** - Cornell University, ***Ann Hajek*** - Cornell University, ***Joseph Hotchkiss*** - Cornell University, ***Stephen Wraight*** - USDA-ARS

MC-5 **Conserving and using entomopathogenic fungi and nematodes within Chile.** ***Andrés France*** - INIA Quilamapu, ***Merino Loreto*** - INIA Quilamapu, ***Edgington Steve*** - CABI, ***Dave Moore*** - CABI, ***Marcos Gerding*** - INIA Quilamapu

MC-6 **Aphid lethal paralysis virus, a potential biological control agent.** ***Liljana Georgievska*** - Iowa State University, ***Sijun Liu*** - Iowa State University, ***W. Allen Miller*** - Iowa State University, ***Bryony Bonning*** - Iowa State University

MC-7 **Entomopathogenic fungus *Myriangium* sp.: biology and possibilities for mass-production.** ***Svetlana Gouli*** - University of vermont, ***Bruce Parker*** - University of Vermont, ***Margaret Skinner*** - University of Vermont, ***Vladimir Gouli*** - University of Vermont

MC-8-STU **Identifying potential microbial control agents in Ghana (rationalising pesticide use against heteropteran pests).** ***Nick Jessop*** - Imperial College, ***Roy Bateman*** - Imperial College

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MC-9 **Influence of polyoxyethylene-(3)-isotridecyl ether as a spreader on the degradation of two species of aphid cuticles by *Beauveria bassiana* SFB-205 supernatant** *Jae-Su Kim* - Entomology Research Laboratory, University of Vermont & Dongbu HiTek, ***Jae-Su Kim*** - Agricultural Research Institute, Dongbu HiTek Co., Ltd., ***Jong Yul Roh*** - School of Agricultural Biotechnology, Seoul National University, ***Yeon Ho Je*** - School of Agricultural Biotechnology, Seoul National University

MC-10 *withdrawn*

MC-11 **The biofumigant effects of *Muscodor albus* on potato tubermoth, *Phthorimaea operculella*, and codling moth, *Cydia pomonella*.** *Lerry Lacey* - USDA, Agricultural Research Service

MC-12 **Monitoring on resistance of brown planthopper (*Nilaparvata lugens*) to imidacloprid and buprofezin** *Jianhong Li* - College of Plant Science and Technology, ***Liang Yao*** - College of Plant Science and Technology, ***Chunhua Qin*** - College of Plant Science and Technology, ***Wei Xu*** - General Station of Hubei Plant Protection, China, ***Kaixiong Zhang*** - General Station of Hubei Plant Protection, China, ***Chuanhua Peng*** - General Station of Hubei Plan Protection, China, ***Hangang Luo*** - General Station of Hubei Plan Protection, China, ***Shengqiao Wang*** - General Station of Hubei Plant Protection, China

MC-13 **Selection of Chilean entomopathogenic fungal strains to yellowjacket wasps.** *Loreto Merino* - INIA Quilamapu, ***Andrés France*** - INIA Quilamapu, ***Marcos Gerding*** - INIA Quilamapu

MC-14 **Conidial mass production of entomopathogenic fungi and tolerance of mass-produced conidia to UV-B radiation and heat.** *Drauzio E. N. Rangel* - Utah State University, ***Éverton K. K. Fernandes*** - Utah State University, ***Helen G. Bignayan*** - Bureau of Plant Industry, Philipinnes, ***Hernani G. Golez*** - Bureau of Plant Industry, Philipinnes, ***Donald W. Roberts*** - Utah State University

MC-15-STU **Evaluation on virulence of *Metarhizium* strains against green peach aphid *Myzus persicae*.** *Le-tian Shan* - Institute of Microbiology, Zhejiang University, ***Ming-guang Feng*** - Institute of Microbiology, Zhejiang University

MC-16 **Isolation and characterization of entomopathogenic fungi for the pine sawyer, *Monochamus saltuarius* Gebler.** *Tae-Young Shin* - Chungbuk National University, ***Sung-Min Bae*** - Chungbuk National University, ***Jae-Bang Choi*** - Chungbuk National University, ***Hyun-Na Koo*** - Chungbuk National University, ***Soo-Dong Woo*** - Chungbuk National University

MC-17 **Host specificity of microsporidia pathogenic to the gypsy moth, *Lymantria dispar* (l.): field studies in Slovakia.** *Leellen Solter* - Illinois Natural History Survey, University of Illinois, ***Daniela Pilarska*** - Bulgarian Academy of Sciences, Institute of Zoology, ***Michael McManus*** - USDA Forest Service, ***Milan Zubrik*** - Forest Research Institute, ***Jan Patocka (deceased)*** - Forest Research Institute, ***Wei-Fone Huang*** - Illinois Natural History Survey, University of Illinois, ***Julius Novotny*** - Forest Research Institute

MC-18 **On-farm control of the leaf miner, *Liriomyza trifolii* in cut flowers using *Isaria fumosorosea*.** *Vitalis Wekesa* - Indian River Research Center, University of Florida, IFAS, ***Pasco Avery*** - Indian River Research Center, University of Florida, IFAS, ***Cindy McKenzie*** - U.S. Horticultural Research Laboratory, ARS-USDA, ***Charles Powell*** - Indian River Research Center, University of Florida, IFAS, ***Lance Osborne*** - Mid-Florida Research and Education Center, University of Florida, IFAS

MC-19 **Evaluation of biological baits for paper wasp *Polistes dominulus* control.** ***Marcos Gerding*** - INIA Quilamapu, *Loreto Merino* - INIA Quilamapu, ***Andrés France*** - INIA Quilamapu, ***Lindsay Barrios*** - INIA Quilamapu

MC-20 **Evaluation of biological baits for paper wasp *Polistes dominulus* control.** ***Vladimir Gouli*** - University of Vermont, ***Margaret Skinner*** - University of Vermont, ***Bruce Parker*** - University of Vermont, *Svetlana Gouli* - University of Vermont, ***Cheryl Frank*** - University of Vermont

MC-21 **Improved production of conidia *in vitro* by *Cordyceps militaris*.** ***In-Pyo Hong*** - National Academy of Agricultural Science, *Richard A. Humber* - USDA-ARS BIological IPM Research

MC-22 **Isolation of the cluster *thuabcdefg* for thuringiensin biosynthesis in *Bacillus thuringiensis* strain ct-43.** ***Xiaoyan Liu*** - Huazhong Agricultural University, ***Lifang Ruan*** - Huazhong Agricultural University, ***Donghai Peng*** - Huazhong Agricultural University, ***ZIniu Yu*** - Huazhong Agricultural University, *Ming Sun* - Huazhong Agricultural University

MONDAY PM

MC-23 **Evaluating the potential use of an entomopathogenic fungus to control the *Cycad aulacaspis* scale in Florida.** ***Veronica Manrique*** - University of Florida, ***Jose Castillo-Altamirano*** - University of Florida, ***Pasco Avery*** - University of Florida, ***Ronald Cave*** - University of Florida

MC-24 **Development and evaluation of *Isaria fumosorosea* for management of Asian citrus psyllid in Texas dooryard citrus.** ***Patrick J. Moran*** - USDA-ARS, BIRU, ***H. Enrique Cabanillas*** - USDA-ARS, BIRU, ***Mark A. Jackson*** - USDA-ARS, NCAUR, ***Christopher A. Dunlap*** - USDA-ARS, NCAUR, ***Pasco B. Avery*** - UF-IFAS Indian River Research and Education Center, ***Wayne B Hunter*** - USDA-ARS, ***David G. Hall*** - USDA-ARS, ***John J. Adamczyk*** - USDA-ARS

MC-25 **Conidial pigmentation protects DNA from UB-B induced damage in the entomopathogenic fungus *Metarhizium anisopliae*.** ***Érika Nascimento*** - Universidade de São Paulo, ***Everaldo Marques*** - Universidade de São Paulo, ***Ludmilla Tonani*** - Universidade de São Paulo, ***Donald Roberts*** - Utah State University, ***Gilberto Braga*** - Universidade de São Paulo

MC-26 **Survival of fungal isolates active against pear thrips in Vermont sugar maple forests.** ***Bruce Parker*** - University of Vermont, ***Svetlana Gouli*** - University of Vermont, ***Vladimir Gouli*** - University of Vermont, ***Margaret Skinner*** - University of Vermont, ***Jae Su Kim*** - University of Vermont

MC-27 **Efficient and eco-friendly recombinant baculovirus insecticide.** ***Hee Jin Shim*** - Department of Agricultural Biotechnology, Seoul National University, ***Jae Young Choi*** - Research Institute for Agriculture and Life Sciences, Seoul National University, ***Yong Wang*** - Department of Agricultural Biotechnology, Seoul National University, ***Jong Yul Roh*** - Department of Agricultural Biotechnology, Seoul National University, ***Soo Dong Woo*** - College of Agriculture, Life and Environment Sciences, Chungbuk National University, ***Byung Rae Jin*** - College of Natural Resources and Life Science, Dong-A University, ***Yeon Ho Je*** - Department of Agricultural Biotechnology, Seoul National University

NEMATODES

N-1 ***Rhabditis* (*oscheius*) species (Nematoda: Rhabditidae), associate with *Agrilus planipennis*.** ***George Kyei-Poku*** - Great Lakes Forestry Centre, Canadian Forest Service, ***Debbie Gauthier*** - Great Lakes Forestry Centre, Canadian Forest Service, ***Kirsty Wilson*** - Great Lakes Forestry Centre, Canadian Forest Service, ***Kees vanFrankenhuyzen*** - Great Lakes Forestry Centre, Canadian Forest Service, ***Johny Shajahan*** - Great Lakes Forestry Centre, Canadian Forest Service

N-2-STU **Survey of entomopathogens attacking larval western corn rootworm *Diabrotica virgifera virgifera* in Iowa cornfields.** ***Melissa Rynerson*** - Iowa State University, ***Aaron Gassmann*** - Iowa State University

N-3 **Directional movement of entomopathogenic nematodes in response to electrical current.** ***David Shapiro-Ilan*** - USDA-ARS, SEFTNRL, ***James Campbell*** - USDA-ARS, GMPRC, ***Ed Lewis*** - UC Davis, ***Daniel Kim-Shapiro*** - Wake Forest University

N-4 **Pest and host plant effects of Ditera, a biopesticide used to control *Meloidogyne incognita*.** ***Kenneth Spence*** - University of California at Davis, Nematology, ***Ed Lewis*** - University of California at Davis, Nematology

N-5 **Host range and production of *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) isolated from Ukraine.** ***Tatyana Stefanovska*** - National University of Life and Environmental Science, ***Harry K Kaya*** - University of California, Department of Nematology

N-6-STU **Influence of *Steinernema carpocapsae* on the native nematode community in pistachio orchards.** ***Amanda K. Hodson*** - University of California-Davis, ***Edwin E. Lewis*** - University of California-Davis, ***Joel Siegel*** - USDA/ARS, SJVASC

17:30 - 19:00	**DINNER BUFFET**	*Outdoor Pavillion*
19:00 - 20:00	**Division Business Meetings**	
	Bacteria Division	*Kokpelli Ballroom III*
	Fungus Division	*White Pine I*
	Microsporidia Division	*Arrowhead I-II*
	Nematode Division	*White Pine II*
	Virus Division	*Kokopelli Ballroom II*

Microsporidia Division Workshop — Monday, 20:00 - 21:00, Painted Horse I-II

Staining Techniques Used for Microsporidia Infecting Invertebrates

Organizer: Dorte Goertz

20:00 **35** **Localisation of microsporidia in amphipod hosts using *in situ* hybridization.** ***Aurore Dubuffet*** - University of Leeds, ***Judith Smith*** - University of Leeds, ***Alison Dunn*** - University of Leeds

20:20 **36** **Fluroescence staining for improved detection of microsporidian spores.** ***Andreas Linde*** - University of Applied Sciences, ***Thomas Kolling*** - University of Applied Sciences

20:40 **37** **Mitochondria related structures, staining of *Nosema* spp.** ***Yi-chun Tsai*** - National Taiwan University, ***Chung-Hsiung Wang*** - National Taiwan University

Virus Division Workshop — Monday, 20:00 - 21:00 — Arrowhead I-II

Advances in Invertebrate Cell Culture

Organizers: Just Vlak, Dwight Lynn

20:00 **38** **Tom Grace and his invaluable contribution to insect cell culture** *Just Vlak* - Wageningen University

20:20 **39** **Use of cell cultures in the study of compounds with insect midgut action.** *Guy Smagghe* - Ghent University

20:40 **40** **Replication of *Agrotis segetum* granulovirus in continuous insect cell lines.** *Dwight E. Lynn* - INSell Consulting, ***Jan W.M. van Lent*** - Laboratory of Virology, Wageningen University, ***Monique M. van Oers*** - Laboratory of Virology, Wageningen University, ***Just M. Vlak*** - Laboratory of Virology, Wageningen University

TUESDAY — 18 August

06:30 - 08:00 **BREAKFAST BUFFET** *Outdoor Pavilion*

07:00 - 12:30 **Registration Open** *Kokopelli Lobby*

Plenary Symposium — Tuesday, 08:00 - 10:00 — Kokopelli Ballroom II-III

The Host-Pathogen Dance: Interactions between Insect Hosts and Their Pathogens

Moderator: Rosalind James

Organizers: Rosalind James, Donald Roberts

08:00 **41** **Dancing with alternate partners: The evolution of virulence factors in insect pathogenic fungi** *Michael Bidochka* - Brock University, ***Nemat Keyhani*** - University of Florida

08:40 **42** **Poldnaviruses as symbionts and immunosuppressive pathogens of insects** *Michael Strand* - University of Georgia

09:20 **43** ***Bt* resistance management mambos nos. 1 and 2.** *Bruce Tabashnik* - Department of Entomology, University of Arizona, ***David Crowder*** - Dept of Entomology, University of Arizona, ***Yves Carrière*** - Dept of Entomology, University of Arizona, ***Aaron Gassmann*** - Department of Entomology, Iowa State University, ***Luke Masson*** - Biotechnology Research Institute, National Research Council of Canada, ***Alejandra Bravo*** - Instituto de Biotecnologia, Universidad Nacional Autónoma de México, ***Mario Soberón*** - Instituto de Biotecnologia, Universidad Nacional Autónoma de México

10:00 - 10:30 **BREAK** *Kokopelli Lobby*

Symposium (Bacterial Division) — Tuesday, 10:30 - 12:10 — Kokopelli Ballroom II-III

Bt Resistance in the Real World

Organizers/Moderators: Bill Moar, Neil Crickmore

10:30 **44** **What is resistance? Coming up with practical and realistic definitions of resistance to *Bt* crops** *Timothy Dennehy* - Monsanto Company, ***Graham Head*** - Monsanto Company

11:00 **45** **How to measure, monitor, and evaluate *Bt* resistance in corn insect pests** *Blair Siegfried* - University of Nebraska, ***Terence Spencer*** - University of Nebraska

11:30 **46** **Delaying *Bt* resistance development in the field** *William Moar* - Monsanto Company

Symposium (Fungus Division) — Tuesday, 10:30 - 12:15 — Painted Horse I-II

Fungi in Soil Habitats—Doing it in the Dirt

Organizer/Moderator: Stefan Jaronski

10:30 **47** **Distribution patterns of fungal entomopathogens in soil habitats** *Nicolai V. Meyling* - University of Copenhagen, Department of Agriculture and Ecology

11:00 **48** **It's a jungle out there! Abiotic and biotic factors affecting entomopathogenic fungi in the soil arena** *Stefan Jaronski* - USDA ARS

11:30 **49** **Efficacy vs. soil insects: tales from the battlefront (successes, failures, & thoughts about why)** *Jarrod Leland* - Novozymes Biologicals, ***Stefan Jaronski*** - USDA-ARS, ***Denny Bruck*** - USDA-ARS Horticultural Crops Research Laboratory

12:00 **50-STU** **The occurrence of entomopathogenic fungi from South African citrus soils** *Tarryn Anne Goble* - Rhodes University, South Africa, ***Joanna Dames*** - Rhodes University, South Africa, ***Martin Hill*** - Rhodes University, South Africa

CONTRIBUTED PAPERS — Tuesday, 10:30 - 11:45 — White Pine I-II

Virus I

Moderators: Kelli Hoover, Peter Krell

10:30 **51** **Viral-encoded fibroblast growth factor enhances virus spread via protease-mediated remodeling of basal lamina** ***John Means*** - Kansas State University, ***A. Lorena Passarelli*** - Kansas State University

10:45 **52** **Targeted immunosuppression: a tool to study the host response to baculovirus infection** *Nor Chejanovsky* - Agricultural Research Organization, ***Hadassah Rivkin*** - Agricultural research Organization, ***Bruce A. Webb*** - University of Kentucky, ***Sassan Asgari*** - School of Integrative Biology

11:00 **53** **Establishment of an insect cell clone that harbors a partial baculoviral genome and is resistant to homologous virus infection.** ***Qingbei Weng*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, ***Kai Yang*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, ***Wei Xiao*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, ***Meijin Yuan*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, *Yi Pang* - State Key Laboratory of Biocontrol, Sun Yat-sen University

11:15 **54-STU** **Novel *cpgv* isolates: deciphering the molecular mechanism involved in overcoming *cpgv* resistance in the codling moth** *Karolin Eberle* - DLR Rheinpfalz, ***Johannes A. Jehle*** - DLR Rheinpfalz

11:30 **55** **The role of the *PI3K-Akt* signal transduction pathway in AcMNPV infection of *Spodoptera frugiperda* cells.** ***Wei Xiao*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, ***Yi Yang*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, ***Qingbei Weng*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, *Kai Yang* - State Key Laboratory of Biocontrol, Sun Yat-sen University, ***Yi Pang*** - State Key Laboratory of Biocontrol, Sun Yat-sen University

11:45 **56-STU** **Cellular secretion induced by ACMNPV replication alters permissibility of cells for narrow host-range baculovirus replication** *Xin-Hua Cheng* - Miami University, ***Lihua Wang*** - Miami University, ***Tamer Salem*** - Miami University, ***Xiao-Wen Cheng*** - Miami University

12:30 - 16:00 **EXCURSION - Utah Olympic Park**
Meet in Grand Summit Lobby to board busses and pick up boxed lunches

17:00 - 18:30 **5K Fun Run/Walk**
Red Pine Lodge (ride the gondola)

19:00 - 23:00 **BBQ AND AUCTION**
Red Pine Lodge (ride the gondola)

WEDNESDAY — 19 August

06:30 - 08:00 **BREAKFAST BUFFET** *Outdoor Pavilion*

07:00 - 12:00 **Registration Open** *Kokopelli Lobby*

Symposium (Virus Division) Wednesday, 08:00 - 10:00
Kokopelli Ballroom II

Invertebrate Antiviral Response

Organizer/Moderator: Nor Chejanovsky

08:00 **57** **Mechanism of RNAi-mediated viral immunity in plants and invertebrates** *Shou-Wei Ding* - Department of Plant Pathology and Microbiology, University of California, Riverside

08:30 **58** **Dicer-2 mediated antiviral response in *Drosophila*** *Jean-Luc Imler* - CNRS-UPR9022, ***Safia Deddouche*** - CNRS-UPR9022, ***Cordula Kemp*** - CNRS-UPR9022, ***Stefanie Muller*** - CNRS-UPR9022, ***Aidan Budd*** - EMBL

09:00 **59** **Alphavirus-derived small RNAs modulate pathogenesis in disease vector mosquitoes** *Kevin Myles* - Virginia Tech, ***Michael Wiley*** - Virginia Tech, ***Elaine Morazzani*** - Virginia Tech, ***Zach Adelman*** - Virginia Tech

09:30 **60** ***Wolbachia*-mediated antiviral protection in insects** *Karyn Johnson* - School of Biological Sciences

CONTRIBUTED PAPERS Wednesday, 08:00 - 10:00
White Pine I-II

Bacteria II

Moderator: David Pauron

61 *withdrawn*

08:00 **62-STU** **An alfa-amylase is a receptor for *Bacillus thuringiensis* Cry4ba toxin in the malaria vector mosquito *Anopheles albimanus*** *Maria Teresa Fernandez-Luna* - Instituto de Biotecnologia, UNAM, ***Sarjeet Gill*** - University of California, Riverside, ***Alejandra Bravo*** - Instituto de Biotecnologia, UNAM, ***Mario Soberón*** - Instituto de Biotecnologia, UNAM, ***Juan Miranda-Rios*** - Instituto de Biotecnologia, UNAM

08:15 **63** ***Aedes aegypti* cadherin serves as a putative receptor of the Cry11aa toxin from *Bacillus thuringiensis* subsp. *sections*** *Jianwu Chen* - Department of Cell Biology and Neuroscience, University of California, ***Karlygash Aimanova*** - Department of Cell Biology and Neuroscience, University of California, ***Claudia Martinez*** - Instituto de Biotecnología, Universidad Nacional Autónoma de México, ***Mario Soberón*** - Instituto de Biotecnología, Universidad Nacional Autónoma de México, ***Sarjeet Gill*** - Department of Cell Biology and Neuroscience, University of California

08:30 **64** **Immunohistochemical analyses of *Bacillus thuringiensis* toxin-binding proteins in gypsy moth larval gut tissue sections** *Algimantas Valaitis* - USDA Forest Service, ***Daniel Krofcheck*** - Ohio Wesleyan University

WEDNESDAY AM

08:45 **65** **Residues of domain III of Cry1ab toxin from *Bacillus thuringiensis* involved in toxicity and receptor binding** Josue Ocelotl - Instituto de Biotecnologia, UNAM, ***Teresa Martinez*** - Instituto de Biotecnologia, UNAM, ***Ivan Arenas*** - Instituto de Biotecnologia, UNAM, ***Sabino Pacheco*** - Instituto de Biotecnologia, UNAM, ***Ricardo Grande*** - Instituto de Biotecnologia, UNAM, ***Alejandra Bravo*** - Instituto de Biotecnologia, UNAM, ***Isabel Gómez*** - Instituto de Biotecnologia, UNAM, ***Mario Soberón*** - Instituto de Biotecnologia, UNAM

09:00 **66** **Cry anti-toxins: A dominant negative phenotype demonstrating that oligomerization is fundamental step in toxin mode of action.** ***Claudia Rodríguez-Almazán*** - Instituto de Biotecnologia, UNAM, ***Liliana Pardo-López*** - Instituto de Biotecnologia, UNAM, ***Carlos Muñoz-Garay*** - Instituto de Biotecnologia, UNAM, ***Luke Masson*** - Biotechnology Research Institute, National Research Council of Canada, ***Mario Soberón*** - Instituto de Biotecnologia, UNAM, ***Alejandra Bravo*** - Instituto de Biotecnologia, UNAM

09:15 **67** **Improvement of Cry toxin insecticidal activity by directed evolution** ***Volker Heinrichs*** - Athenix, ***Jayme Williams*** - Athenix

09:30 **68** **Expression of *Bacillus thuringiensis israelensis* Cry10aa toxin: parasporal body formation, toxicity and synergism with Cyt1AA** ***Alejandro Hernández-Soto*** - Centro de Investigación en Biología Celular y Molecular, ***M. Cristina Del Rincón-Castro*** - División de Ciencias de la Vida, ***Ana M. Espinoza*** - Centro de Investigación en Biología Celular y Molecular, ***Jorge E. Ibarra*** - CINVESTAV-Irapuato

CONTRIBUTED PAPERS — Wednesday, 08:00 - 10:00 — Painted Horse I-II

Fungi I

Moderator: Helen Roy

08:00 **69** **Pathogenicity of the entomopathogenic fungi *Beauveria bassiana* and its effect on the behavior of *Polistes dominulus* wasp** ***Andrés France*** - INIA Quilamapu, ***Loreto Merino*** - INIA Quilamapu, ***Marcos Gerding*** - INIA Quilamapu, ***Ricardo Ceballos*** - INIA Quilamapu

08:15 **70** **Weakness in the band: nutrient imbalance and immunodeficiency in mass-migrating cannibalistic katydids** ***Robert Srygley*** - USDA-Agriculture Research Service, ***Patrick Lorch*** - Kent State University

08:30 **71** **A *Beauveria bassiana*-based "trap and kill" device to control the major Chagas disease vector in southern South America** ***Nicolas Pedrini*** - INIBIOLP (CCT La Plata CONICET-UNLP), ***Juan R. Girotti*** - INIBIOLP (CCT La Plata CONICET-UNLP), ***M. Patricia Juarez*** - INIBIOLP (CCT La Plata CONICET-UNLP)

08:45 **72** ***Pandora* infections in *Formica* ants** ***David P. Hughes*** - Museum of Comparative Zoology, Harvard University, ***Jørgen Eilenberg*** - Centre for Social Evolution, Department of Agriculture and Ecology, University of Copenhagen, ***Jacobus J. Boomsma*** - Centre for Social Evolution, Department of Biology, University of Copenhagen, ***Annette B. Jensen*** - Centre for Social Evolution, Department of Agriculture and Ecology, University of Copenhagen

09:00 **73** **Overlapping niches of *Beauveria bassiana* in a conifer forest** ***Helen Roy*** - Centre for Ecology & Hydrology, ***Emma Ormond*** - Anglia Ruskin University, ***Judith Pell*** - Rothamsted Research, ***Alison Thomas*** - Anglia Ruskin University

09:15 **74** **Effects of the peptide mycotoxin destruxin a on the renal tubules of *Rhodnius prolixus*** ***Esau Ruiz-Sanchez*** - Department of Biology, University of Toronto at Mississauga, ***Ian Orchard*** - University of Toronto at Mississauga, ***Angela Lange*** - University of Toronto at Mississauga

09:30 **75-STU** **Hydrophobins of the entomopathogenic fungus *Beauveria bassiana*** ***Shizhu Zhang*** - University of Florida, ***Nemat Keyhani*** - University of Florida

09:45 **76** **A selective medium for isolating entomopathogenic fungi *Metarhizium* and *Beauveria* from Western United States soil** ***Éverton K. K. Fernandes*** - Utah State University, ***Chad A. Keyser*** - Utah State University, ***Drauzio E. N. Rangel*** - Utah State University, ***R. Nelson Foster*** - USDA/APHIS/PPQ/CPHST Lab, ***Donald W. Roberts*** - Utah State University

10:00 - 10:30 **BREAK** *Kokopelli Lobby*

Symposium (Bacterial Division) — Wednesday, 10:30 - 12:10 — Kokopelli Ballroom II

Bt the Bacterium, Ecology and Infection

Organizer/Moderator: Christina Nielson-LeRoux

10:30 **77** ***Bacillus thuringiensis: in vivo* development, plasmid conjugation and expression of virulence and adaptation factors** ***Christina Nielsen-LeRoux*** - INRA, ***Nadine Daou*** - INRA, ***Fuping Song*** - CASS, ***Clelton Santos*** - Dept. Biologia Geral, ***Gyslaine Vilas-Boas*** - Dept. Biologia Geral, ***Christophe Buisson*** - INRA, ***Jie Zhang*** - Institute of Plant Protection, CAAS, Beijing, China, ***Olivia Arantes*** - Universidad Estadual de Londrina, Brazil, ***Didier Lereclus*** - INRA

WEDNESDAY AM

11:00 **78** ***Bacillus thuringiensis* toxins as cooperative public goods--can social evolution theory explain the dynamics of *Bt* virulence**
Ben Raymond - University of Oxford

11:30 **79** **The role of mid-gut flora in the pathogenicicity of *Bacillus thuringiensis* to susceptible and resistant Lepidoptera** ***Paul R. Johnston*** - Department of Biochemistry, School of Life Sciences, ***Ben Raymond*** - Mathematical Ecology Research Group, Dept of Zoology, ***Vidisha Krishnan*** - Department of Biochemistry, School of Life Sciences, *Neil Crickmore* - Department of Biochemistry, School of Life Sciences, ***Denis J. Wright*** - Division of Biology, Faculty of Natural Science, ***Richard J. Ellis*** - Molecular Pathogenesis and Genetics, Veterinary Laboratories Agency, UK, ***Michael B. Bonsall*** - Mathematical Ecology Research Group, Dept of Zoology, University of Oxford, UK

12:00 **80** ***Bacillus thuringiensis*, resident gut microbiota, and innate immunity in lepidopteran insects** *Jo Handelsman* - UW-Madison, Dept. of Bacteriology, ***Kenneth Raffa*** - UW-Madison, Dept. of Entomology, ***Nichole Broderick*** - Global Health Institute

CONTRIBUTED PAPERS — Wednesday, 10:30 - 12:30, Painted Horse I-II

Nematodes II

Moderator: Bishwo Adhikari

10:30 **81-STU** ***Thripinema fuscum* parasitism reduces the vector competency of *Frankliniella fusca* to transmit tomato spotted wilt virus**
Kelly Sims - Department of Entomology and Nematology, ***Joseph Funderburk*** - North Florida Research and Education Center, ***Stuart Reitz*** - Center for Medical, Agricultural and Veterinary Entomology, ***Drion Boucias*** - Department of Entomology and Nematology

10:45 **82-STU** **Aggregation behavior in adults of *Steinernema carpocapsae*** *Yolanda Reyes* - Centro de Investigación en Alimentación y Desarrollo, A.C., ***Ali Asaff*** - Centro de Investigación en Alimentación y Desarrollo, A.C., ***Mayra De la Torre*** - Centro de Investigación en Alimentación y Desarrollo, A.C.

11:00 **83-STU** **Rate of lateral dispersal of the entomopathogenic nematode *Heterorhabditis bacteriophora* from infected host cadavers in soil** *Harit K. Bal* - Department of Entomology, OARDC, The Ohio State University, Wooster, OH 44691, ***Robin R.A.J. Taylor*** - Department of Entomology, OARDC, The Ohio State University, Wooster, OH 44691, ***Parwinder S. Grewal*** - Department of Entomology, OARDC, The Ohio State University, Wooster, OH 44691

11:15 **84** **Tritrophic trade-offs affecting growth and resistance to pathogens for a polyphagous herbivore.** *Aaron Gassmann* - Iowa State University, ***Patricia Stock*** - University of Arizona, ***Bruce Tabashnik*** - University of Arizona, ***Michael Singer*** - Wesleyan University

11:30 **85** **Soil microarthropod response to the application of *Steinernema carpocapsae*–killed insects in maize and refuge habitats.** *Randa Jabbour* - Washington State University, ***Mary Barbercheck*** - Pennsylvania State University

11:45 **86** **Entomopathogenic nematodes in Tanzania.** *Solveig Haukeland* - Bioforsk, Norwegian Institute for Agricultural and Environmental Research, ***Suma Mwaitulo*** - Sokoine University of Agriculture, ***Anne Laudisoit*** - CERVA-CODA, ***May-Guri Saethre*** - Bioforsk, Norwegian Institute for Agricultural and Environmental Research, ***Amon Maerere*** - Sokoine University of Agriculture, ***Khuong Nguyen*** - University of Florida, Entomology and Nematology Dept

12:00 **87-STU** **Isolation and characterization of a North American species of *Phasmarhabditis*.** *Nathaniel Keplinger* - Department of Biology, Brigham Young University, ***Byron Adams*** - Department of Biology, Brigham Young University

CONTRIBUTED PAPERS — Wednesday, 10:30 - 12:30, White Pine I-II

Microbial Control I

Moderator: Sunday Ekesi

10:30 **88-STU** **Heat-induced post-stress growth delay: a biological trait of many *Metarhizium* isolates that may reduce field efficacy.** *Chad A. Keyser* - Department of Biology, Utah State University, ***Éverton K. K. Fernandes*** - Department of Biology, Utah State University, ***Stefan T. Jaronski*** - USDA ARS NPARL, ***Donald W. Roberts*** - Department of Biology, Utah State University

89 *withdrawn*

WEDNESDAY AM

10:45 **90-STU** **Can *Steinernema sp.* be lured by an environmental bacterium? A case study with new isolates from Tanzania .** *Anne Laudisoit* - Veterinary and Agrochemical Research Centre, ***Solveig Haukeland*** - Norwegian Institute for Agricultural and Environmental Research, ***Pierre Wattiau*** - Veterinary and Agrochemical Research Centre

11:00 **91** **Identification and blend effects of repellent volatiles of entomopathogenic fungi towards the termite *Macrotermes michaelseni*.** ***David M. Mwangi*** - International Centre of Insect Physiology and Ecology (icipe), *Nguya Kalemba Maniania* - International Centre of Insect Physiology and Ecology (icipe), ***Ahmed Hassanali*** - International Centre of Insect Physiology and Ecology (icipe), ***Peter A. Njagi*** - International Centre of Insect Physiology and Ecology (icipe), ***Linus M. Gitonga*** - Jomo Kenyatta University of Agriculture and Technology

11:15 **92-STU** **Influences of local and long-distance dispersal on the evolution of *Bt* resistance in cabbage looper populations.** *Michelle Franklin* - Dept. of Zoology, University of British Columbia, ***Judith Myers*** - Dept. of Zoology, University of British Columbia, ***Carol Ritland*** - Dept. of Forest Sciences, University of British Columbia

CONTRIBUTED PAPERS — Wednesday, 10:30 - 12:30 — Kokopelli Ballroom III

Virus II

Moderators: Rollie Clem, Jim Slavicek

10:30 **93** **Polydnaviruses as organelles– An alternative paradigm.** *Brian Federici* - Department of Entomology, ***Yves Bigot*** - Laboratoire d'Estude des Parasites Genetiques

10:45 **94-STU** **Proteomic analysis of Chilo iridescent virus particles.** *Ikbal Agah Ince* - Wageningen University and Research Centrum, ***Sjef A. Boeren*** - Wageningen University and Research Centrum, ***Monique M. van Oers*** - Wageningen University and Research Centrum, ***Jacques J.M. Vervoort*** - Wageningen University and Research Centrum, ***Just M. Vlak*** - Wageningen University and Research Centrum

11:00 **95** **Analysis of MDSGHV transcripts in *Spodoptera frugiperda* cells and in house flies.** *Tamer Salem* - University of Florida, ***Alejandra Garcia-Maruniak*** - University of Florida, ***Verena-Ulrike Lietze*** - University of Florida, ***James Maruniak*** - University of Florida, ***Drion Boucias*** - University of Florida

11:15 **96** **Four major envelope proteins of white spot syndrome virus bind to form a complex.** *Yipeng Qi* - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Qing Zhou*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Yan Zhu*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Feng Yan*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University

11:30 **97** **Molecular analysis of protein kinase gene (*amv197*) of *Amsacta moorei* entomopoxvirus.** Hacer Muratoglu - Karadeniz Technical University, ***Remziye Nalcacioglu*** - Karadeniz Technical University, *Zihni Demirbag* - Karadeniz Technical University

11:45 **98-STU** **The sfav1a p64 basic virion protein and its homologues comprise a novel family of viral genome condensing proteins.** *Tatsinda Spears* - Graduate Program in Cell, Molecular, and Developmental Biology, University of California, Riverside, ***Dennis K. Bideshi*** - California Baptist University, ***Jeffrey J. Johnson*** - Department of Entomology, University of California, Riverside, ***Yeping Tan*** - Department of Entomology, University of California, Riverside, ***Yves Bigot*** - Laboratorie d'Estude des Parasites Genetiques, ***Brian A. Federici*** - Department of Entomology, and Graduate Programs in Genetics and Cell, Molecular and Developmental Biology, University of California, Riverside

12:00 **99** **Role of cellular microRNAs in ascovirus infection.** Mazhar Hussain - University of Queensland, *Sassan Asgari* - University of Queensland

12:15 **100** ***Solenopsis invicta* virus 3, a new positive-strand RNA virus infecting the red imported fire ant.** *Steven Valles* - USDA-ARS

12:30 - 14:00 **LUNCH BUFFET** *Outdoor Pavillion*

12:30 - 14:00 **STUDENT WORKSHOP & LUNCH**
How to Get a Postdoc Position and Get Into the Scientific Network
Painted Horse I-II

12:40 **101** **Life after the PhD: Find your own work-life balance.** *Agata Jakubowska* - University of Valencia

13:05 **102** **From passion to profession: An unconventional journey from high school teacher to research scientist.** *Pasco B. Avery* - UF/IFAS/Indian River Research and Education Center

13:30 **103** **There's more than one way to skin a cat: Finding your path to a professorship.** *Richard Plunkett* - New Mexico Highlands University

WEDNESDAY PM

Symposium (Fungus Division) Wednesday, 14:00 - 16:00
Kokopelli Ballroom III

Insect Defense Responses to Fungal Pathogens

Moderators: Tariq Butt, Drion Boucias
Organizers: Tariq Butt, Rosalind James

14:00 **104** **Invertebrate antifungal immunity – Finding avenues for exploitation.** Miranda Whitten - Swansea University, ***Norman Ratcliffe*** - Swansea University, ***Tariq Butt*** - Swansea University, ***Drion Boucias*** - University of Florida, Gainesville, USA

14:30 **105** **The *Drosophila melanogaster* model to study fungal and bacterial infections : Novel insights into insect host defense.** Marie Gottar - UPR 9022 du CNRS, ***Nadine Nehme*** - UPR 9022 du CNRS, ***Stefanie Limmer*** - UPR9022 du CNRS, ***Samuel Liegeois*** - UPR9022 du CNRS, ***Dominique Ferrandon*** - UPR9022 du CNRS, ***Alexei Matskevitch*** -, ***Jessica Quintin*** - Richard Bou Aoun, Arshad Ayyaz, Philippe Giammarinaro, Shane Cronin, J. Andrew Pospisilik, Daniel Schramek, Ricardo de Matos Simoes, Ingo Ebersberger, Arndt von Haeseler, Josef Penninger

15:00 **106** **Transcriptome analysis of honey bee, *Apis mellifera* larvae infected with chalkbrood fungus.** ***Katherine Aronstein*** - USDA/ARS, ***Dan Murray*** - USDA/ARS

15:30 **107** **Immunity related genes expressed in the alfalfa leafcutting bee, *Megachile rotundata*.** ***Junhuan Xu*** - Utah State University, Department of Biology, ***Rosalind James*** - USDA-ARS, Pollinating Insects Biology, Management and Systematics

CONTRIBUTED PAPERS Wednesday, 14:00 - 16:00
White Pine I-II

Microbial Control II

Moderator: Steven Arthurs

14:00 **108** **Compatibility of *Bacillus thuringiensis* Cry1 and Vip3a proteins for resistance management in *Spodoptera frugiperda*.** ***Janete A.D. Sena*** - University of Valencia, ***Carmen Sara Hernández-Rodríguez*** - University of Valencia, ***Juan Ferré*** - University of Valencia

14:15 **109-STU** **Identification and characterization of *Bacillus* species toxic to several mosquito species.** ***Sabrina Hayes*** - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University, ***Hyun-Woo Park*** - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University, ***Michael Hudon*** - Indian River Mosquito Control District

14:30 **110-STU** **The dose-transfer chain: improving the lab-to-field process in cocoa.** ***Nick Jessop*** - Imperial College, ***Godfred Awudzi*** - Cocoa Research Institute of Ghana, ***Roy Bateman*** - Imperial College

14:45 **111-STU** **Utilization of entomopathogenic fungi for the control of plant pathogenic fungi.** ***Sastia Prama Putri*** - International Center for Biotechnology, Osaka University, ***Hiroshi Kinoshita*** - International Center for Biotechnology, Osaka University, ***Fumio Ihara*** - National Institute of Fruit Tree Science, ***Yasuhiro Igarashi*** - Biotechnology Research Center, Toyama Prefectural University, ***Takuya Nihira*** - International Center for Biotechnology, Osaka University

15:00 **112** **Characterization of *Beauveria bassiana* isolates associated with *Agrilus planipennis* populations in Michigan.** Louela Castrillo - Cornell University, ***Leah Bauer*** - USDA Forest Service, ***Houping Liu*** - Michigan State University, ***Michael Griggs*** - USDA ARS, ***John Vandenberg*** - USDA ARS

15:15 **113-STU** **Storage conditions affect intensity of delayed germination in *Beauveria bassiana* and *Metarhizium anisopliae* conidia.** Marcos Faria - Cornell University, ***Mark Ramos*** - USDA-ARS, ***Ann Hajek*** - Cornell University, ***Stephen Wraight*** - USDA-ARS

15:30 **114** **Production of microsclerotia of *Metarhizium anisopliae* using deep-tank, liquid fermentation.** ***Mark Jackson*** - USDA-ARS-NCAUR, ***Stefan Jaronski*** - USDA-ARS-PARL

15:45 **115** **Influence of carbohydrates on the control efficacy of the entomopathogenic fungus *Beauveria bassiana*.** ***Jeong Jun Kim*** - Applied Entomology Division, ***Seon Heo*** - Applied Entomology Division, ***Yujin Song*** - Applied Entomology Division, ***Chang-Gyu Park*** - Applied Entomology Division, ***Hong-Hyun Park*** - Applied Entomology Division

CONTRIBUTED PAPERS Wednesday, 14:00 - 16:00
Kokopelli Ballroom II

Virus III

Moderators: Basil Arif, Zhihong Hu

14:00 **116** **Defining the response of *Mamestra brassicae* to mixed infections.** ***Helen Hesketh*** - NERC Centre for Ecology and Hydrology, ***Claus Svendsen*** - NERC Centre for Ecology and Hydrology, ***Rosie Hails*** - NERC Centre for Ecology and Hydrology

WEDNESDAY PM

14:15 **117-STU** **Mixed infections of wild-type and fast-acting recombinant variants in insect-baculovirus pathosystems.** *Liljana Georgievska* - Wageningen University, Laboratory of Virology, ***Wopke Wopke van der Werf*** - Wageningen University, Centre for Crop Systems Analysis, ***Mark P. Zwart*** - Wageningen University, Laboratory of Virology, ***Kelli Hoover*** - Pennsylvania State University, Department of Entomology, ***Jenny Cory*** - Department of Biological Sciences, ***Just M. Vlak*** - Wageningen University, Laboratory of Virology

14:30 **118** **Expression of *Helicoverpa armigera* chitin deacetylase-like protein improves nucleopolyhedrovirus speed of kill.** *Agata Jakubowska* - University of Valencia, ***Silvia Caccia*** - University of Valencia, ***Karl Gordon*** - CSIRO Entomology, ***Juan Ferré*** - University of Valencia, ***Salvador Herrero*** - University of Valencia

14:45 **119** **Tissue tropism of the *Musca domestica* salivary gland hypertrophy virus.** *Verena-Ulrike Lietze* - Entomology and Nematology Department, University of Florida, ***Tamer Z. Salem*** - Entomology and Nematology Department, University of Florida, ***Pannipa Prompiboon*** - Entomology and Nematology Department, University of Florida, ***Drion Boucias*** - Entomology and Nematology Department, University of Florida

15:00 **120** **Geographical distribution of *Musca domestica* salivary gland hypertrophy virus that infects and sterilizes female house flies.** *Pannipa Prompiboon* - University of Florida, ***Verena-Ulrike Lietze*** - University of Florida, ***Tamer Salem*** - University of Florida, ***Drion Boucias*** - University of Florida

15:15 **121** **Strategies to control salivary gland hypertrophy virus (SGHV) infection in tsetse laboratory colonies.** *Adly Abd-Alla* - Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, ***Andrew Parker*** - Entomology Unit, ***FAO/IAEA Agriculture and Biotechnology Laboratory,*** ***Max Bergoin*** - Laboratoire de Pathologie Comparée, Université Montpellier II, France, ***Just Vlak*** - Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands, ***Marc Vreysen*** - Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory

16:00 - 16:30 BREAK *Kokopelli Lobby*

Wednesday, 16:30 - 17:30
Kokopelli Ballroom I

POSTERS 2

Posters will be displayed from Wednesday 8:00 to Thursday 13:30.

BENEFICIAL INVERTEBRATES

BI-1 **Hytrosaviridae: A proposal for classification and nomenclature of a new insect virus family.** *Adly Abd-Alla* - Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory

BI-2 **Bee cell cultures: (*Apis mellifera*, Apidae: Hymenoptera.)** *Wayne Hunter* - USDA,ARS

BI-3-STU **Molecular dectection of *Noesma bombi* and *Crithidaia bombi* in wild and commercial populations of bumble bees in the U.S.** *Anna Morkeski* - University of Massachusetts - Amherst, ***John Burand*** - Department of Plant, Soil and Insect Science, ***Anne Averill*** - University of Massachusetts – Amherst

BI-4 **Identification and characterization of a new microsporidium isolated from the silkworm, *Bombyx mori*** *Liangen Shi* - College of Animal Sciences, Zhejiang University

BI-5 **Presence and prevalence of viruses in local and migratory honeybees in Massachusetts.** Anna Welch - University of Massachusetts – Amherst, *John Burand* - University of Massachusetts – Amherst

FUNGI

F-1 *withdrawn*

F-2-STU **Gene expression analysis of *Beauveria bassiana* during infection of *Spodoptera exigua*.** *Duriya Chantasingh* - Mahidol University, ***Kusol Pootanakit*** - Mahidol University, ***Nemat Keyhani*** - University of Florida, ***Lily Eurvilaichitr*** - National Center for Genetic Engineering and Biotechnology

F-3 **Identification of a hybrid PKS-NPRS required for the biosynthesis of NG-391 and NG-393 metabolites in *Metarhizium anisopliae*.** *Bruno Donzelli* - USDA, ARS, Bio-IPM Research Unit, ***Stuart Krasnoff*** - USDA, ARS, Bio-IPM Research Unit, ***Alice Churchill*** - Dept. of Entomology, ***John Vandenberg*** - USDA, ARS, Bio-IPM Research Unit, ***Donna Gibson*** - USDA, ARS, Bio-IPM Research Unit

F-4-STU **Effect of water activity and nitrogen source on *Isaria fumosorosea* propagule characteristics.** *Francisco Escobar Herrera* - CIAD A.C., ***Veronica Mata*** - CIAD A.C., ***Ali Asaff*** - CIAD A.C.

F-5 ***Beauveria bassiana*, *Metarhizium anisopliae*, and *M. anisopliae* var. *acridum* conidia: Tolerance to imbibitional damage.** Marcos Faria - Cornell University, ***Ann Hajek*** - Cornell University, *Stephen Wraight* - USDA-ARS

WEDNESDAY PM

F-6 **Biology of Mormon Cricket *Anabrus simplex* and laboratory-colony development.** *Rodrigo B. Ferreira* - Utah State University, ***Éverton K. K. Fernandes*** - Utah State University, ***Chad A. Keyser*** - Utah State University, ***Scott Treat*** - Utah State University, ***Donald W. Roberts*** - Utah State University

F-7 **Genotypic diversity of *Beauveria bassiana* isolates in acridids from the Northern Plains of the United States.** *Stefan Jaronski* - USDA ARS, ***Paul Kaufmann*** - USDAARS, ***John Gaskin*** - USDA ARS

F-8 **Genome profiling in hybrid strains of entomopathogenic fungi *Lecanicillium* spp. (*Verticillium lecanii*).** *Masanori Koike* - Obihiro University, ***Fusako Kaibara*** - Kyushu University, ***Daigo Aiuchi*** - Obihiro University, ***Yoshinori Hatakeyama*** - College of Bioresource Sciences, Nihon University, ***HIdetoshi Iwano*** - College of Bioresource Sciences, Nihon University

F-9 **A yeast-like symbiont of *Tenebrio molitor* and *Zophobas atratus*.** *Jeff Lord* - USDA-ARS

F-10 **Immune response of Mormon crickets to infection by *Beauveria bassiana*.** *Robert Srygley* - USDA-Agriculture Research Service, ***Stefan Jaronski*** - USDA-Agriculture Research Service

F-11 **Statistical considerations in the analysis of data from replicated bioassays.** *Stephen Wraight* - USDA-ARS, ***Stefan Jaronski*** - USDA-ARS, ***Mark Ramos*** - USDA-ARS, ***Michael Griggs*** - USDA-ARS, ***John Vandenberg*** - USDA-ARS

F-12 **Overexpression of manganese superoxide dismutase from *Beauveria bassiana* enhances its tolerance to oxidative stress.** *Xue-qin Xie* - Institute of Microbiology, Zhejiang University, ***Sheng-hua Ying*** - Institute of Microbiology, Zhejiang University, ***Ming-guang Feng*** - Institute of Microbiology, Zhejiang University

F-13 **Thioredoxin from the entomopathogenic fungi, *Beauveria bassiana*: Gene cloning, characterization and functional expression.** *Sheng-hua Ying* - Institute of Microbiology, Zhejiang University, ***Xiao-hui Wang*** - Institute of Microbiology, Zhejiang University, ***Ming-guang Feng*** - Institute of Microbiology, Zhejiang University

F-14 **Study of temperature-growth interactions of entomopathogenic fungi isolated from chalk grassland in the UK.** Emma Turner - Centre for Ecology and Hydrology, *Helen Hesketh* - Centre for Ecology and Hydrology, ***Rosie Hails*** - Centre for Ecology and Hydrology

MICROSPORIDIA

M-1 **Predation by the carabid beetle, *Calosoma sycophanta* affects transmission of microsporidia infecting gypsy moth larvae.** *Dörte Goertz* - University of Natural Resources and Applied Life Sciences, ***Gernot Hoch*** - Resources and Applied Life Sciences

M-2 **Temperature and simulated rain affect horizontal transmission of the microsporidium *Nosema lymantriae*.** *Gernot Hoch* - University of Natural Resources and Applied Life Sciences, ***Sieglinde Pollan*** - Resources and Applied Life Sciences, ***Carina Steyer*** - Resources and Applied Life Sciences, ***Dörte Goertz*** - Resources and Applied Life Sciences

M-3 **High temperature eliminates microsporidia from an insect host.** *Johny Shajahan* - Illinois State University, ***Austin Omer*** - Illinois State University, ***William Newgent*** - Illinois State University, ***Katriona Elmer*** - Illinois State University, ***Douglas W. Whitman*** - Illinois State University, ***Regina Stoerger*** - Dept. of Biology, Illinois State University, ***Will Hatch*** - Dept. of Biology, Illinois State University

M-4-STU **Characterization of a microsporidia isolated from S. *litura* and its relationship with a nucleopolyhedrovirus in Vietnam.** *Thao Le Thi Thanh* - Student, ***Madoka Nakai*** - Associate Professor, ***Yoshinori Hatakeyama*** - Research Assistant, ***Iwano Hidetoshi*** - Professor, ***Yasuhisa Kunimi*** - Professor

M-5 **New microsporidia-host association: four new species infecting bark lice (Psocoptera).** *Yuliya (Julia) Sokolova* - Institute of Cytology Russian Academy of Sciences, ***Igor Sokolov*** - Louisiana State University AgCenter Entomology Dept., ***Christopher Carlton*** - Louisiana State University AgCenter Entomology Dept.

VIRUS

V-1-STU **Cloning and recombinant expression of glycoprotein e2 of the classical Swine Fever virus.** *Sung-Min Bae* - Chungbuk National University, ***Hyun-Na Koo*** - Chungbuk National University, ***Yeon-Ho Je*** - Seoul National University, ***Byung-Rae Jin*** - Dong-A University, ***Soo-Dong Woo*** - Chungbuk National University

V-2 **Further studies on divalent cations and transmission of CUNIDVB in *Culex quinquefasciatus*.** *James Becnel* - USDA/ARS/CMAVE

WEDNESDAY PM

V-3 **Proviral structure and organization of *Cotesia congregata* bracovirus.** *Annie Bezier* - IRBI CNRS Université de Tours, ***Gabor Gyapay*** - CEA Genoscope, ***Georges Periquet*** - IRBI CNRS Université de Tours, ***Elisabeth Herniou*** - IRBI CNRS Université de Tours, ***Jean-Michel Drezen*** - IRBI CNRS Université de Tours

V-4 *withdrawn*

V-5 **Systemic pathogenesis of ACMNPV budded virus in *Anticarsia gemmatalis* larvae.** *Eric Haas-Stapleton* - California State University, Long Beach, ***Alisa De La Cruz*** - California State University, Long Beach, ***Elisa Martinez*** - California State University, Long Beach, ***Marianne Torres*** - California State University, Long Beach, ***Tiffany Chen*** - California State University, Long Beach

V-6 **Sequence and analysis of the genome of the Illinois isolate of *Agrotis ipsilon* multiple nucleopolyhedrovirus.** *Robert Harrison* - Invasive Insect Biocontrol and Behavior Laboratory, USDA Agricultural Research Service

V-7 ***In vivo* pathogenesis of *Lymantria dispar* M Nucleopolyhedrovirus using a VP39-GFP/HSP70/LACZ recombinant.** *Kelli Hoover* - Penn State University, ***James Slavicek*** - U.S. Forest Service, ***Nancy Hayes-Plazolles*** - U.S. Forest Service, ***James McNeil*** - Penn State University

V-8 **Leafhopper infecting rhabdovirus: Taastrup-like virus.** *Wayne Hunter* - USDA-ARS, ***Scott Adkins*** - USDA-ARS

V-9 **Iridescent virus infection in glassy-winged sharpshooter (*Homalodisca vitripennis*: Hemiptera).** *Wayne Hunter* - USDA-ARS, ***Ute Albrecht*** - USDA-ARS

V-10 **Viral pathogens in leafhopper vectors of Pierce's disease.** *Wayne Hunter* - USDA-ARS, ***Mizuri Marutani-Hert*** - USDA-ARS, ***Laura Hunnicutt*** - North Carolina State University

V-11 **Bioaerosols: Insect transmitted pathogens.** *Wayne Hunter* - USDA,ARS, ***Cindy McKenzie*** - USDA,ARS, ***Bailey Mitchell*** - Electrostatic Space Charge Systems, LLC

V-12 **Metagenomics of leafhoppers and psyllidspsyllids: Discovery of bacteriophages.** *Wayne Hunter* - USDA,ARS, ***Robert Shatters, Jr.*** - USDA, ARS

V-13 **Enhancing the multiplication of NuclepHPH optimization.** *Agata Jakubowska* - University of Valencia, ***Juan Ferré*** - University of Valencia, ***Salvador Herrero*** - University of Valencia

V-14-STU **Production of a full length cDNA clone and infectious transcripts of deformed wing virus.** *Seth Levy* - University of Massachusetts A– Amherst, ***Woojin Kim*** - University of Massachusetts – Amherst, ***John Burand*** - University of Massachusetts – Amherst

V-15 **Improved procedures for identification of Orcytes nudivirus disease in the Pacific region.** *Sean Marshall* - AgResearch, ***Jana Monk*** - AgResearch, ***Shareen Prasad*** - Secretariat of the Pacific Community, ***Nicola Richards*** - AgResearch, ***Trevor Jackson*** - AgResearch

V-16 **Melanized encapsulation and apoptosis impact baculovirus infection in gypsy moth (*Lymantria dispar*).** *James McNeil* - Penn State University, ***Matthew Gardner*** - Penn State Unversity, ***Diana Cox-Foster*** - Penn State Unversity, ***Kelli Hoover*** - Penn State University

V-17 ***In vivo* inhibition of HZ-2V pathology using anti P74 RNAi.** *Justin Nguyen* - University of Massachusetts - Amherst, ***Woojin Kim*** - University of Massachusetts – Amherst, ***John Burand*** - University of Massachusetts – Amherst

V-18-STU **Generation of BMNPV- resistant BMN cells.** *Daisuke Ohtsuka* - Laboratory of applied molecular entomology, Graduate School of Agriculture, Hokkaido university, ***Ken Sahara*** - Laboratory of applied molecular entomology, Graduate School of Agriculture, Hokkaido university, ***Shin-ichiro Asano*** - Laboratory of applied molecular entomology, Graduate School of Agriculture, Hokkaido university, ***Hisanori Bando*** - Laboratory of applied molecular entomology, Graduate School of Agriculture, Hokkaido university

V-19-STU **Analysis of ACMNPV immediate-early gene knockout viruses – with a focus on the function of *ME53*.** *Chikako Ono* - Graduate School of Agriculture, Hokkaido University, ***Shin-ichiro Asano*** - Graduate School of Agriculture, Hokkaido University, ***Ken Sahara*** - Graduate School of Agriculture, Hokkaido University, ***Hisanori Bando*** - Graduate School of Agriculture, Hokkaido University

V-20 **Protein expression profiles of permissive, semipermissive and non-permissive cells infected by baculovirus.** *Holly Popham* - USDA ARS BCIRL, ***Cynthia Goodman*** - USDA ARS BCIRL, ***James Grasela*** - USDA ARS BCIRL

WEDNESDAY PM

V-21 **Functional analysis of the inhibitor of apoptosis genes in antheraea pernyi nucleopolyhedrovirus.** *Yipeng Qi* - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Songya LV*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Feng Yan*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Hua Xu*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Daqin Zhang*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University

V-22 **Recombinant expression of the structural proteins of *Porcine* reproductive and respiratory syndromevirus using BMNPV.** *Soo-Dong Woo* - Chungbuk National University, ***Jeong-Mi Oh*** - Chungbuk National University, ***Hyun-Na Koo*** - Chungbuk National University, ***Yeon-Ho Je*** - Seoul National University, ***Byung-Rae Jin*** - Dong-A University

V-23-STU **Host RNA polymerase-dependent transcription strategies of insect viruses.** *Jianli Xue* - Miami University, ***WeiYing Wang*** - Miami University, ***Colin Turney*** - Miami University, ***Xiao-Wen Cheng*** - Miami University

V-24 **A recombinant *Anticarsia gemmatalis* multiple nucleopolyhedrovirus with interruption of the *pif-1* gene.** Briana ***Cardoso Ferreira*** - Embrapa Recursos Genéticos e Biotecnologia, ***Maria Elita Batista de Castro*** - Embrapa Recursos Genéticos e Biotecnologia, Francisco José Rivera Pinedo - ***Faculdade Juscelino*** Kubitschek, *Marlinda Lobo de Souza* - Embrapa Recursos Genéticos e Biotecnologia

V-25 **Direct fusion is an alternative pathway for ACMNPV to efficiently gain entry into insect and mammalian cells.** ***Sicong Dong*** - Wuhan Institute of Virology, CAS, ***Manli Wang*** - Wuhan Institute of Virology, CAS, ***Fei Deng*** - Wuhan Institute of Virology, CAS, ***Zhihong Hu*** - Wuhan Institute of Virology, CAS, *Hualin Wang* - Wuhan Institute of Virology,CAS

V-26 **The CPGV spindlin-like protein enhanced the infectivity of baculoviruses and *Bacillus thuringiensis*. *Xiangyang Liu*** - Wuhan Institute of Virology, Chinese Academy of Sciences, *Xiulian Sun* - Wuhan Institute of Virology, Chinese Academy of Sciences

V-27 **Metagenomics approach to discover virus: *Diaphorina citri* reovirus.** ***Mizuri Marutani-Hert*** - USDA-ARS, *Wayne Hunter* - USDA-ARS, ***David Hall*** - USDA-ARS

V-28 **An active DNA photolyase (AMV025) from *Amsacta moorei* entomopoxvirus.** ***Remziye Nalcacioglu*** - Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, ***Kazim Sezen*** - Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, ***Ikbal Agah Ince*** - Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, ***Just M. Vlak*** - Laboratory of Virology, Wageningen University, ***Zihni Demirbag*** - Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, ***Basil Arif*** - Great Lakes Forestry Centre, Canadian Forest Service, *Monique M. van Oers* - Laboratory of Virology, Wageningen University

V-29 **Bacterial but not baculoviral infections stimulate hemolin expression in *Helocoverpa zea* and *Heliothis virescens*. *Olle Terenius*** - Global and Swedish Research Cooperation, *Holly Popham* - USDA ARS BCIRL, ***Kent Shelby*** - USDA ARS BCIRL

V-30 **Integration of picorna-like viruses in multiple insect taxa.** ***Danielle Tufts*** - University of Texas at Tyler, ***K Spencer*** - University of Texas at Tyler, *Wayne Hunter* - USDA-ARS, ***Daymon Hail*** - University of Texas at Tyler, ***Blake Bextine*** - University of Texas at Tyler

V-31 **Identification of a nucleopolyhedrovirus in winter moth populations from Massachusetts.** ***Anna Welch*** - University of Massachusetts - Amherst, George Boettner - University of Massachusetts – Amherst, ***Vincent D'Amico*** - USDA- Forest Service, University of Delaware, ***Joseph Elkinton*** - University of Massachusetts - Amherst, *John Burand* - University of Massachusetts - Amherst

V-32 **Physiological basis for increased AGIPMNPV infection following feeding of *Agrotis ipsilon* larvae on Herculex I corn.** ***Nina Schmidt*** - Iowa, ***Jessica Haywood*** - Iowa State University, *Bryony Bonning* - Iowa State University

17:30 - 19:00 **DINNER BUFFET** *Outdoor Pavillion*

19:00 - 20:00 **Division Business Meetings**
Microbial Control Division *Kokpelli Ballroom II*
Pathogens of Beneficial Invertebrates Division *White Pine I-II*

Beneficial Division Workshop Wednesday, 20:00 - 21:15
White Pine I-II

Bee Health—Diseases and Cures

Organizer/Moderator: Elke Genersch

20:00 **122** **RNAi at work in real life application: Targeting invertebrate pests and beneficial organisms' diseases.** *Eyal Ben-Chanoch* - Beeologics, ***Nitzan Paldi*** - Beeologics

20:15 **123** **Parasites in bumble bees - An overview of virulence, epidemiology and the impact of commercial breeding.** *Mark Brown* - Royal Holloway, University of London

20:30 **124** **Honey bee viruses and their impact on honey bee health.** *Elke Genersch* - Institute for Bee Research

20:45 **125** **Chalkbrood distribution and transmission in U.S. populations of the alfalfa leafcutting bee.** *Rosalind James* - USDA-ARS Bee Biology & Systematics Lab, ***Theresa Pitts-Singer*** - USDA-ARS Bee Biology & Systematics Lab, ***Ellen Klinger*** - USDA-ARS Bee Biology & Systematics Lab

21:00 **126** **The prevalence, distribution and hosts of *Crithidia bombi* in wild bumble bee populations.** *James Strange* - USDA-ARS-Pollinating Insect Research Unit, ***Nils Cordes*** - University of Illinois, Illinois Natural History Survey, ***Leellen Solter*** - University of Illinois, Illinois Natural History Survey, ***Terry Griswold*** - USDA-ARS-Pollinating Insect Research Unit, ***Sydney Cameron*** - Department of Entomology, and Program in Ecology, Evolution and Conservation Biology, University of Illinois

THURSDAY — 20 August

6:30 - 08:00 **BREAKFAST BUFFET** *Outdoor Pavillion*

07:00 - 12:00 **Registration Open** *Kokopelli Lobby*

Symposium (Microbial Control Division) Thursday, 08:00 - 10:00 White Pine I-II

Biopesticides in Organic Farming: Available and Potential Technologies

Organizer/Moderator: Surendra Dara

08:00 **127** **Microbial control of agricultural pests in South Korea.** *Jeong Jun Kim* - Applied Entomology Division, NAAS, RDA, ***Sang Guei Lee*** - Applied Entomology Division, NAAS, RDA, ***Siwoo Lee*** - Applied Entomology Division, NAAS, RDA, ***Hyeong-Jin Jee*** - Agricultural Microbiology Division

08:30 **128** **Dipel® biological insecticide: An integral tool for pest control in organic apple production.** *Russ Eldridge* - Valent BioSciences

09:00 **129** **Entrust® insecticide: A key tool in organic farming.** *Tom Meade* - Dow AgroSciences, LLC, ***Jesse Richardson*** - Dow AgroSciences, LLC, ***Luis Gomez*** - Dow AgroSciences, LLC, ***James E. Dripps*** - Dow AgroSciences, LLC, ***Doris Paroonagian*** - Dow AgroSciences, LLC

09:30 **130** **Baculoviruses and fungi as commercial biopesticides: Poised for a breakthrough?** *Michael Dimock* - Certis USA

Symposium (Microsporidia Division) Thursday, 08:00 - 10:00 Painted Horse I-II

Microsporidia of Beneficial Arthropods

Organizer/Moderator: David Oi

08:00 **131** ***Nosema ceranae* research in Spain: A review** *Raquel Martín-Hernández* - Bee Pathology Laboratory, Apicultural Center of Marchamalo, ***Aránzazu Meana*** - Animal Health Dep. Faculty of Veterinary (UCM), ***Pilar García-Palencia*** - Medicine and Surgery Dep. Faculty of Veterinary (UCM), ***Mariano Higes*** - Bee Pathology Laboratory, Apicultural Center of Marchamalo

08:24 **132** ***Nosema ceranae* and nosema disease in honey bee.** *Wei-Fone Huang* - Illinois Natural History Survey, University of Illinois, ***Leellen Solter*** - Illinois Natural History Survey, University of Illinois, ***Chung-Hsiung Wang*** - Department of Entomology, National Taiwan University

08:48 **133** **Are microsporidia involved in bumble bee decline?** *Nils Cordes* - Illinois Natural History Survey, University of Illinois, ***Leellen Solter*** - Illinois Natural History Survey, University of Illinois, ***Sydney Cameron*** - Department of Entomology, University of Illinois, ***Jeffrey Lozier*** - Department of Entomology, University of Illinois, ***James Strange*** - USDA-ARS Pollinating Insect Research Unit, ***Terry Griswold*** - USDA-ARS Pollinating Insect Research Unit

09:12 **134** **Social parasitism in microsporidia: *Kneallhazia solenopsae* development in fire ant colonies.** *Yuliya (Julia) Sokolova* - Institute of Cytology Russian Academy of Sciences, ***James Fuxa*** - Louisiana State University AgCenter Entomology Dept.

09:36 **135** **Microsporidia of lady beetles used for biological pest control.** ***Taro Saito*** - Saint Mary's University, ***Susan Bjornson*** - Saint Mary's University

CONTRIBUTED PAPERS Thursday, 08:00 - 10:00 Kokopelli Ballroom III

Virus IV

Moderators: Just Vlak, Lorena Passarelli

08:00 **136** **Identification of proteins associated with *Autographa californica* nucleopolyhedrovirus budded virions.** ***Ranran Wang*** - Wuhan Institute of Virology, CAS, ***Fei Deng*** - Wuhan Institute of Virology, CAS, ***Lin Guo*** - Wuhan University, ***Hualin Wang*** - Wuhan Institute of Virology, CAS, ***Zhihong Hu*** - Wuhan Institute of Virology, CAS

THURSDAY AM

08:15 **137-STU** **ACMNPV *me53* is a non-essential gene required for efficient budded virus production.** *Jondavid de Jong* - University of Guelph, ***Basil Arif*** - Great Lakes Forestry Centre, ***David Theilmann*** - Pacific Agri-food Research Centre, ***Peter Krell*** - University of Guelph

08:30 **138** **Majority of the f proteins from granuloviruses are functional analogues of *gp64* of ACMNPV.** ***Feifei Yin*** - Wuhan Institute of Virology,CAS, ***Manli Wang*** - Wuhan Institute of Virology,CAS, ***Fei Deng*** - Wuhan Institute of Virology,CAS, ***Zhihong Hu*** - Wuhan Institute of Virology,CAS, *Hualin Wang* - Wuhan Institute of Virology,CAS

08:45 **139** **Identification of protein-protein interactions of the ODV associated proteins of HEARNPV.** *Ke Peng* - Wuhan Institute of Virology,CAS, ***Mingzhi Wu*** - Wuhan Institute of Virology,CAS, ***Fei Deng*** - Wuhan Institute of Virology,CAS, ***Hualin Wang*** - Wuhan Institute of Virology,CAS, ***Zhihong Hu*** - Wuhan Institute of Virology,CAS

09:00 **140** **The core gene *ac96* of AVMNPV encodes a *per os* infectivity factor (*pif-4*).** ***Minggang Fang*** - Pacific Agri-Food Research Centre, *Yingchao Nie* - Plant Science, Faculty of Land and Food Systems, ***Stephanie Harris*** - Saskatoon Research Centre, ***Martin Erlandson*** - Saskatoon Research Centre, ***David Theilmann*** - Pacific Agri-Food Research Centre

09:15 **141-STU** **On the binding and fusion of Baculovirus ODV to midgut epithelial cells: Distribution and orientation of *pif* proteins.** *Ke Peng* - Laboratory of Virology, ***Jan W.M. van Lent*** - Laboratory of Virology, ***Monique M. van Oers*** - Laboratory of Virology, ***Zhihong Hu*** - Wuhan Institute of Virology, ***Just M. Vlak*** - Laboratory of Virology

09:30 **142** **Fuctions of *cis*-elements of *Helicoverpa armigera* nucleopolyhedroviruses *p13* gene in early expression modulation.** *Yipeng Qi* - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Songya LV*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Yingle Liu*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Nan Lu*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, ***Enqi Du*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University

09:45 **143-STU** **A novel gene (*orf74*) found in the *Maruca vitrata* nucleopolyhedrovirus.** *Shih-Chia Yeh* - Entomology, National Taiwan University, ***Chih-Yu Wu*** - Entomology, National Taiwan University, ***Chu-Fang Lo*** - Zoology, National Taiwan University, ***Chung-Hsiung Wang*** - Zoology, National Taiwan University

10:00 - 10:15 **BREAK** *Kokopelli Lobby*

10:15 - 12:30 **SIP Annual Business Meeting**
Kokopelli Ballroom II-III

Retired SIP Members: Where are our old friends now? *Elizabeth W. Davidson* - Arizona State University

12:00 - 13:30 **LUNCH BUFFET** *Outdoor Pavillion*

12:00 - 13:30 **STUDENT BUSINESS MEETING & LUNCH** *Doc's*

12:00 - 13:30 **STUDENT AWARDS COMMITTEE MEETING & LUNCH**
The Cabin Restaurant

Symposium (Virus Division) Thursday, 13:30 - 15:30
Kokopelli Ballroom III

The Viral Face of PDV's: Origin and Structure of the Chromosomally Integrated PDV Genomes

Organizers/Moderators: Jean-Michel Drezen, Sassan Asgari

13:30 **144** **Phylogenomic approaches unravel the origin and tempo of bracovirus evolution.** *Elisabeth Herniou* - IRBI - UMR CNRS 6035, ***Julien Theze*** - IRBI - UMR CNRS 6035, ***Annie Bezier*** - IRBI - UMR CNRS 6035, ***Georges Periquet*** - IRBI - UMR CNRS 6035, ***Jean-Michel Drezen*** - IRBI - UMR CNRS 6035

14:00 **145** **Genetic changes in bracoviruses associated with host shifts in braconid parasitoid wasps.** *James Whitfield* - University of Illinois, ***Michael Strand*** - University of Georgia

14:30 **146** **Chromosomally integrated glyptapanteles bracovirus genomes: Structure and organization** *Dawn Gundersen-Rindal* - USDA ARS Invasive Insect Biocontrol & Behavior Laboratory, ***Chris Desjardins*** - J. Craig Venter Institute, ***Vish Nene*** - J. Craig Venter Institute

15:00 **147** **A viral origin for ichnovirus particles?** *Anne-Nathalie Volkoff* - INRA, ***Jean-Michel Drezen*** - CNRS, ***Véronique Jouan*** - INRA, ***Serge Urbach*** - Plate-forme Protéomique CNRS UMR 5203, INSERM U661,UM1, UM2, ***Gabor Gyapay*** - CEA-GENOSCOPE-Centre National de Séquençage

Symposium (Nematode Division) Thursday,13:30 - 15:30
Painted Horse I-II

Ecological Interactions in Entomopathogenic Nematodes

Organizers/Moderators: Albrecht Koppenhofer, Harry Kaya

13:30 **148** **Risk-sensitive infection strategies: A new way to look at parasite behavior.** *Edwin Lewis* - Department of Nematology, ***James Campbell*** - USDA, ***Hsieh Fushing*** - Department of Statistics, ***David Shapiro-Ilan*** - USDA, ***Glen Stevens*** - Ferrum College

13:54 **149** **Virulence and infectivity of entomopathogenic nematodes: Changes with age.** *Christine T. Griffin* - National University of Ireland Mayooth, ***Adam G. Guy*** - National University of Ireland Mayooth, ***Denis J. Wright*** - Imperial College London, ***Michael T. Gaffney*** - Teagasc

14:18 **150** **Overcoming antagonists and environmental hazards.** *Claudia Dolinski* - UENF/CCTA/LEF, ***David Shapiro-Ilan*** - USDA-ARS, SEFTNRL

14:42 **151** **Spatio-temporal nematode-host interactions in turfgrass.** *Albrecht Koppenhöfer* - Dept. Entomology, Rutgers University, ***Benjamin McGraw*** - Dept. Golf and Plant Sciences, State Univeristy of New York

15:06 **152** **Ecological dynamics of entomopathogenic nematodes in a natural system.** *Daniel Gruner* - University of Maryland, ***Donald Strong*** - University of California - Davis

CONTRIBUTED PAPERS Thursday, 13:30 - 14:30
White Pine I-II

Microbial Control III

Moderator: Jean Maniania

13:30 **153** **Evaluation of *Iisaria fumosorosea* to control the Asian citrus psyllid, *Diaphorina citri*.** *Steven Arthurs* - Mid-Florida Research and Education Center, ***Pasco Avery*** - Indian River Research and Education Center

13:45 **154** **Efficacy of fungal pathogens as biologically-based agents for control of adult mosquitoes.** *Nancy E. Beckage* - University of California-Riverside, ***Anita R. Gordillo*** - University of California-Riverside

14:00 **155** **Evaluation of entomopathogenic fungi for the management of *Sternochetus mangiferae* on mango.** *Sunday Ekesi* - International Centre of Insect Physiology and Ecology (ICIPE), ***Rabiu Salisu Adamu*** - Ahmadu Bello University, ***Nguya Kalemba Maniania*** - International Centre of Insect Physiology and Ecology (ICIPE)

14:15 **156** **Efficacy of *Bacillus thuringiensis* var. *israelensis* strain ABG-6193 against field-collected larvae of three Culicine mosquito species.** *Samy Hussein Mohamed* - Plant Protection Dept

14:30 **157** **Possible use of *Metarhizium* for controlling the lesser mealworm, *Alphitobius diaperinus* in broiler houses.** ***Galina Gindin*** - The Volcani Center, ***Asayel Rot*** - Kimron Veterinary Institute, ***Avishai Lublin*** - Kimron Veterinary Institute, ***Itamar Glazer*** - The Volcani Center, *Michael Samish* - Kimron Veterinary Institute

14:45 **158** **The effects of a granulovirus infection on the growth and development of *Helicoverpa armigera* larvae.** *Gustav Bouwer* - School of Molecular and Cell Biology, University of the Witwatersrand, ***Garry Coulson*** - School of Molecular and Cell Biology, University of the Witwatersrand

15:00 **159** **Methods for testing side-effects of pesticides on *Neozygites floridana*.** *Vitalis Wekesa* - Departament of Entomology, Plant Pathology and Agricultural Zoology, Escola Superior de Agricultural, ***Markus Knapp*** - Koppert Biological Systems, ***Italo Delalibera*** - Departament of Entomology, Plant Pathology and Agricultural Zoology, Escola Superior de Agricultural

15:30 - 16:00 **BREAK** *Kokopelli Lobby*

Symposium (Cross-Divisional) Thursday, 16:00 - 18:00
Kokopelli Ballroom II

Multitrophic Interactions: Implications for Invertebrate Pathogens

Organizers/Moderators: Jenny Cory, Helen Roy

16:00 **160** **Multitrophic level interactions and population dynamics.** *Saskya van Nouhuys* - University of Helsinki & Cornell University

16:30 **161** **Scared sick? Predator-pathogen facilitation strengthens herbivore suppression.** *Ricardo Ramirez* - Texas A&M University, ***William Snyder*** - Washington State University

17:00 **162** **Multitrophic interactions – Are entomopathogens and parasitoids good for each other?** *Jenny Cory* - Simon Fraser University

17:30 **163** **Interactions involving entomopathogenic fungi and insects: An applied perspective.** *Jason Baverstock* - Rothamsted Research, ***Helen Roy*** - Centre for Ecology and Hydrology, ***Judith Pell*** - Rothamsted Research

CONTRIBUTED PAPERS Thursday, 16:00 - 18:00
White Pine I-II

Microbial Control IV

Moderator: Vitalis Wekesa

THURSDAY PM

16:00 **164** **Broad spectrum potential of the biopesticide, *Isaria fumosorosea* for managing insect pests of citrus.** *Pasco B. Avery* - University of Florida, Institute of Food and Agricultural Sciences, ***Wayne B. Hunter*** - USDA, ARS, ***David G. Hall*** - USDA, ARS, ***Mark A. Jackson*** - USDA, ARS, Crop Bioprotection Research Unit, ***Charles A. Powell*** - University of Florida, Institute of Food and Agricultural Sciences, ***Michael E. Rogers*** - University of Florida, Citrus Research and Education Center

16:15 **165** **Control of pine weevil *Hylobius abietis* with entomopathogenic nematodes, and safety of nematodes to nontarget insects.** *Christine T. Griffin* - National University of Ireland Maynooth, ***Aoife B. Dillon*** - Coillte, ***Darragh Ennis*** - National University of Ireland Maynooth, ***Khalil M. Alameen*** - National University of Ireland Maynooth, ***Aileen Foster*** - National University of Ireland Maynooth, ***Chris D. Harvey*** - National University of Ireland Maynooth

16:30 **166** **Field efficacy of *Beauveria bassiana* on the *Vespula germanica* wasp nests.** *Loreto Merino* - NIA Quilamapu, ***Andrés France*** - NIA Quilamapu, ***Marcos Gerding*** - NIA Quilamapu, ***Ricardo Ceballos*** - NIA Quilamapu

16:45 **167** **Persistence and efficacy of entomopathogens in potting media.** *Anne Nielsen* - University of California at Davis, ***Kenneth Spence*** - University of California at Davis, ***Denny Bruck*** - USDA-ARS, ***Edwin Lewis*** - University of California at Davis

17:00 **168** **Field efficacy of a baculovirus isolate that doesn't cause the liquefaction of *Spodoptera frugiperda* dead larvae.** *Fernando Valicente* - Embrapa, ***Corina Macedo*** - Student, ***José Wolff*** - Universidade Presbiteriana Mackenzie, ***Edmar Tuelher*** - Student-Embrapa, ***Carlos Paiva*** - Student-Embrapa, ***Alan Costa*** - Student-Embrapa

17:15 **169** **Successful introduction of Green Muscle® into Madagascar for the control of the migratory locust *Locusta migratoria* Capito . *Jocelyn J.H. Rajaonarison*** - FOFIFA, *Nguya Kalemba Maniania* - International Centre of Insect Physiology and Ecology (icipe), ***Rahalivavololona Njaka*** - FOFIFA, ***Saholy Ramiliarijaona*** - FOFIFA

17:30 **170** ***Beauveria bassiana* UV resistance in the laboratory and its virulence against the coffee berry borer in the field.** ***Sandra Valdes*** - Cenicafé. National Centre of Coffee Research., *Carmenza E Gongora* - Cenicafe. National Centre of Cofee Research.

17:45 **171** **Biological control of Asian corn borer using *Wolbachia* infected line of *Trichogramma dendrolimi* and its evaluation.** *Bin Cong* - Laboratory for Biological Control, Shenyang Agricultural University, ***Xihua Wang*** - Laboratory for Biological Control, Shenyang Agricultural University, ***Haiyan Zhang*** - Laboratory for Biological Control, Shenyang Agricultural University, ***Haitao Qian*** - Laboratory for Biological Control, Shenyang Agricultural University, ***Hui Dong*** - Laboratory for Biological Control, Shenyang Agricultural University

CONTRIBUTED PAPERS — Thursday, 16:00 - 17:45 — Painted Horse I-II

Bacteria III

Moderator: Juan Luis Jurat-Fuentes

16:00 **172** **Pathogenicity island in the Mexican *Serratia entomophila* mor4.1 active against *Phyllophaga blanchardi* larvae (Coleoptera).** *Maria Eugenia Nuñez-Valdez* - Facultad De Ciencias Agropecuarias, Universidad Autonoma del Estado de Morelos, ***Jean Chen*** - Depts. of Cell Biology and Neuroscience3, University of California, Riverside, ***Sarjeet Gill*** - Depts. of Cell Biology and Neuroscience, University of California, Riverside, ***Francisco Villalobos*** - Facultad De Ciencias Agropecuarias, Universidad Autonoma del Estado de Morelos, ***Zitlhally Rodriguez-Segura*** - Centro de Investigación en Biotecnología2, Universidad Autónoma del Estado de Morelos

16:15 **173** **From insect to man: A functional genomic comparison of clinical and insect pathogenic strains of *Photorhabdus*. *Maria Sanchez-Contreras*** - University of Bath, ***Andrea Dowling*** - University of Exeter, ***Richard ffrench-Constant*** - University of Exeter, *Nick Waterfield* - University of Bath

16:30 **174** **The fate of toxin complexes in cultured cells.** *Michelle Hares* - University of Exeter, ***Stewart Hinchliffe*** - University of Exeter, ***Richard ffrench-Constant*** - University of Exeter

16:45 **175** ***Bacillus thuringiensis* biopesticide produced with different amounts of carbon and nitrogen.** *Fernando Valicente* - Embrapa Maize and Sorghum, ***Andre Horta*** - Agronomy Student, ***Lucas Mendes*** - Agronomy Student, ***Amanda Lanza*** - Student, ***Sandra Miranda*** - Student

17:00 **176** **Expression of aminopeptidases in *Ostrinia nubilalis* (Hübner).** ***Maria Cristina Crava*** - Dep. Genética. Universitat de Valencia, ***Yolanda Bel*** - Dep. Genética. Universitat de Valencia, ***Barbara Manachini*** - Dep. Animal Biology. U. Palermo, ***Juan Ferré*** - Dep. Genética. Universitat de Valencia, *Baltasar Escriche* - Dep. Genética. Universitat de Valencia

THURSDAY PM

17:15 **177** **Characterization, distribution and cloning *cry1* genes efficient against fall armyworm, *Spodoptera frugiperda*, in Brazil.** *Fernando Valicente* - Embrapa, ***Edgard Picoli*** - Post Doc / Embrapa, ***Maria Vasconcelos*** - Embrapa, ***Newton Carneiro*** - Embrapa, ***Andrea Carneiro*** - Embrapa - C.P 151, ***Cláudia T. Guimarães*** - Embrapa, ***Ubiraci G. P. Lana*** - Embrapa

17:30 **178** **Plasmid capture system and its applications.** *Jong Yul Roh* - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, ***Yong Wang*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, ***Qin Liu*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, ***Xueying Tao*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Jae ***Young Choi*** - Research Institute for Agriculture and Life Sciences, Seoul National University, Korea, Hee ***Jin Shim*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, ***Hong Guang Xu*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, ***Soo Dong Woo*** - Department of Agricultural Biology Chungbuk National University, Korea, ***Byung Rae Jin*** - College of Natural Resources and Life Science, Dong-A University, Korea, ***Yeon Ho Je*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea

19:00 - 20:00 **COCKTAIL HOUR** *Kokopelli Lobby*

20:00 **BANQUET & AWARDS CEREMONY** *Kokopelli Ballroom*

2009 ABSTRACTS

NOTE

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

-STU Indicates papers being judged for GRADUATE STUDENT presentation awards

100 Indicates abstract number for ORAL presentations

B-10 Indicates abstract number for POSTER presentations

MONDAY — 17 August

Symposium (Virus Division) Monday, 10:30 - 12:30.
Kokopelli Ballroom II

Insect RNA Viruses: Advances and Applications

Organizers/Moderators: Bryony Bonning, Karyn Johnson

Symposium - Monday, 10:30 1

The *Tetraviridae*: Prospects for targeted drug delivery.

Rosemary Dorrington - Rhodes University, Grahamstown, Eastern Cape, South Africa; ***Marli Vlok*** - Rhodes University, Grahamstown, Eastern Cape, South Africa; ***Michele Tomasicchio*** - Rhodes University, Grahamstown, Eastern Cape, South Africa
Address for correspondence: r.dorrington@ru.ac.za

The Tetraviridae are a family of small insect RNA viruses that exclusively infect the midguts of the larvae of five closely related families within the Order Lepidoptera including economically important agricultural pests. Tetravirus particles consist of one or two single-stranded positive sense genomic RNAs encapsidated in an icosahedral capsid of approximately 40 nm in diameter, with a T=4 symmetry. Tetravirus capsids comprise 240 subunits of a single capsid protein precursor that undergoes C-terminal autoproteolytic cleavage during particle maturation. Each subunit is comprised of three regions: the internal helical domain, a beta-barrel and an external immunoglobulin-like domain, which is proposed to function in receptor binding during infection of host cells. Tetravirus virus-like particles (VLPs) self-assemble in insect and yeast cells expressing the virus capsid protein precursor. These VLPs have been reported to package both the mRNA expressing the capsid protein as well as cellular RNAs and there is evidence of encapsidation and delivery of non-viral RNA encoding GFP to larval midgut cells. We have studied the assembly of tetravirus particles, focussing on the factors that regulate particle assembly and RNA encapsidation. This talk will focus on new insights into the mechanism of RNA packaging, the factors required for specific encapsidation of viral RNAs and the potential for developing new technologies for loading tetraviral VLPs with non-viral RNA.

Symposium - Monday, 11:00 2

Assembly of multi-layered viral nanoparticles: A new approach for vaccine design.

Anette Schneemann - The Scripps Research Institute, La Jolla, CA, USA
Address for correspondence: aschneem@scripps.edu

Virus particles are natural nanoparticles that are known for their strong immunogenicity. This property is based on the repetitive, ordered structure of their component proteins, their particulate nature and ability to appropriately stimulate the innate and adaptive immune system. These features are preserved in virus-like particles (VLPs), which represent recombinantly expressed viruses that lack a viral genome and are thus not infectious. VLPs can be used as safe vaccines against the viruses they represent as well as other pathogens. The latter is achieved by genetically inserting into the VLP nano-platform foreign peptides or proteins which are then displayed in the same ordered, repetitive fashion as the viral proteins themselves. We recently showed that the anthrax toxin receptor can be displayed in a multivalent fashion on the surface of VLPs of the insect virus Flock House virus (FHV). The resulting chimeric nanoparticles function as a potent anthrax antitoxin in cell culture and protect rats from lethal toxin challenge. This work is of general importance because it shows that protein domains containing more than 150 amino acids can be displayed on a viral nanoparticle platform in a biologically functional form, suggesting numerous additional applications. The chimeric particles could then be decorated *in vitro* with another protein layer, the anthrax protective antigen (PA). Such multi-layered particles elicited a potent neutralizing antibody response against PA that protects rats from lethal toxin challenge four weeks after a single immunization in the absence of adjuvants. This represents a dramatic improvement over the currently available anthrax vaccine, which requires numerous injections over the course of 18 months. The chimeric VLP platform represents a dually acting reagent for the treatment as well as for protection against anthrax. We are currently developing strategies to expand the utility of this nanoparticle platform for development of vaccines against other pathogens.

Symposium - Monday, 11:30 3

Alphavirus transducing systems.

Ken Olson - Colorado State University, Fort Collins, CO, USA
Address for correspondence: Kenneth.Olson@ColoState.z

Alphavirus transducing systems (ATSs) are important tools for efficient gene expression in mosquitoes and other insects. ATSs are based on mosquito-borne RNA viruses of the genus *Alphavirus (Togaviridae)* and were originally used as non-heritable, virus expression systems to complement transposon-based DNA transformation systems in mosquitoes. ATSs have been engineered to express small proteins such as single chain antibodies, anti-mosquito neurotoxins, anti-bacterial peptides, or RNAi suppressors. In addition, ATSs also have been used for virus-induced gene silencing of dengue viruses, reporter genes, or endogenous genes in adult mosquitoes. Currently we have ATSs that infect *Aedes, Anopheles, Armigeres*, and *Culex* species of mosquitoes as well as *Drosophila melanogaster* and *Bombyx mori*. We have shown that ATSs efficiently express GOIs in midguts, fat body, hemocytes, and salivary glands of different mosquito species. Other uses of ATSs include identifying virus determinants of mosquito infection and in microarray studies of transcription in midguts following infection. In the last five years, new ATSs have been developed that infect A.aegypti (ATSs 5dsMRE16 and 5dsCHIKV), *Anopheles gambiae* (5dsONN), *Culex tritaeniorhynchus* (5dsMRE16), and *Culex tarsalis* (5dsWEEV). We are currently using ATSs to develop transmission models that will allow us to visualize virus transmission from vectors to host. This work represents highly novel approaches for monitoring virus transmission from a mosquito to an animal model and will represent a significant advancement towards defining virus-vector interactions that affect transmission.

Symposium - Monday, 12:00 4

The dicistroviruse: Advances and applications.

Bryony Bonning - Iowa State University, Ames, IA, USA
Address for correspondence: bbonning@iastate.edu

The dicistroviruses are members of a recently defined and rapidly growing family of picornavirus-like RNA viruses. Dicistroviruses are pathogenic to beneficial arthropods such as honey bees and shrimp, and to insect pests of medical and agricultural importance. While significant advances have been made in elucidation of virus particle structure and the remarkable mechanisms of internal ribosome entry in viral RNA, there are large gaps in our understanding of dicistrovirus biology. The recent construction of infectious clones of dicistrovirus genomes may help fill these gaps in knowledge. I will provide an overview of the dicistroviruses with emphasis on recent advances including construction of infectious clones, and activation of the RNA interference pathway for protection

of economically important arthropods from dicistrovirus infection. I will also discuss the potential use of dicistroviruses as biopesticides.

Symposium (Beneficials Division) Monday, 10:30 - 12:30
Painted Horse I-II

Diseases in Populations of Beneficial Invertebrates

Organizer/Moderator: Grant Stentiford

Symposium - Monday, 10:30 5

Crustacean diseases in European legislation: An overview of recent developments.

Grant Stentiford - European Community Reference Laboratory for Crustacean Diseases, Weymouth, Dorset, United Kingdom
Address for correspondence: grant.stentiford@cefas.co.uk

The EC Council Directive 2006/88/EC adopted during 2008 has listed three crustacean diseases: White Spot Disease (WSD) caused by the White Spot Syndrome Virus (WSSV), Yellowhead disease (YHD) caused by Yellowhead Virus (YHV) and Taura syndrome (TS) caused by Taura syndrome Virus (TSV). Their inclusion within the Directive acknowledges a lack of protection (regarding biosecurity) to aquaculture and wild stocks of crustaceans in European waters in previous aquatic animal health legislation and aligns legislation for crustaceans with that already in place for fish and molluscs. The listing recognises the global importance of diseases such as WSD, TS and YHD in causing significant economic losses in farming regions, the lack of control measures available to deal with disease outbreaks should they occur and the potential for their international transfer via trade in live and commodity products. This presentation will provide an overview of the work of the European Community Reference Laboratory for Crustcaean Diseases and wider, the growing requirement for crustacean disease expertise within the European Union. The presentation will be illustrated using several important pathogens of commercially exploited crustaceans from European waters.

Symposium - Monday, 11:00 6

Social insects and their parasites in the wild – What do we know and where should we go?

Mark Brown - Royal Holloway, University of London, Egham, Surrey, United Kingdom
Address for correspondence: mark.brown@rhul.ac.uk

Social insects play a key role in ecosystem processes and thus are beneficial both directly, through their use as commercial pollinators, and indirectly as keystone species. The social Hymenoptera – ants, some bees and some wasps – pose particular problems and advantages to parasites. They represent extremely rich and concentrated populations for parasites to exploit, but their spatial structure, division of labour, and social immunity pose considerable hurdles in comparison to solitary hosts. In this talk I will summarise these issues and discuss what we know about parasites – their epidemiology and impact – in natural populations of social insects

Symposium - Monday, 11:30 7

Fungal Pathogens from the genus *Ascosphaera* in populations of honey bees and solitary bees.

Jørgen Eilenberg - University of Copenhagen, Copenhagen, Denmark; ***Annette B. Jensen*** - University of Copenhagen, Copenhagen, Denmark; ***Rosalind R. James*** - USDA-ARS Pollinating Insects Research Unit, Logan, Utah, USA; ***Bo V. Pedersen*** - University of Copenhagen, Copenhagen, Denmark; ***Svetlana Vojvodic*** - University of Copenhagen, Copenhagen, Denmark; ***Anja A. Wynns*** - University of Copenhagen, Copenhagen, Denmark
Address for correspondence: rosalind.james@ars.usda.gov

Ascosphaera is a unique genus among the insect pathogenic fungi. The infection route is based upon oral ingestion followed by penetration in the gut, as opposed to most insect pathogenic fungi, which infect through the host cuticle. The genus is found solely in associations with bees from Apoidea, either as pathogens infecting the host or as saprotrophs living on pollen provisions or nesting materials. The species from the genus do solely reproduce sexually. The most well known species are those which are pathogenic (causing chalkbrood) on economically important hosts: *Ascosphaera apis* infecting the honey bee *Apis mellifera,* and *A. aggregata* infecting the alfalfa leafcutting bee *Megachile rotundata.* We initiated a range of studies in order to elucidate the fascinating interaction between host and pathogen populations. These include: a) differential susceptibility across honey bee races in larval *A. apis* resistance, b) honey bee drone susceptibility to *A. apis* compared with worker susceptibility, c) genetic diversity of *A. apis* in Danish honey bee colonies, d) mixed infections (*A. apis* and *Aspergillus flavus*) in honey bees, e) the diversity of *Ascosphaera* spp. among solitary cavity-nesting bees. We present novel biological data and discuss the interaction between these fungal pathogens and their host populations.

Symposium - Monday, 12:00 8

Disease transmission in honey bees.

Elke Genersch - Institute for Bee Research, Hohen Neuendorf, Brandenburg, Germany
Address for correspondence: elke.genersch@rz.hu-berlin.de

Honey bees are important pollinators of crops, fruit, and wild flowers. Therefore, they are indispensable for both, a sustainable and profitable agriculture and the maintenance of non-agricultural ecosystems. Honeybees are attacked by numerous pathogens including viruses, bacteria, and fungi causing serious infectious diseases. In addition, different metazoan parasites can infest honeybees. For a given pathogen, its virulence and its major transmission routes (e. g. vertical, horizontal, vectorial) are two factors related to and influencing each other. Disease transmission in the eusocial honey bee is a complex process involving transmission between individual honeybees within a colony on one hand and between bee colonies ('super-organism bee') on the other hand. Using two examples from honey bee pathology we will show the interplay between 'mode of transmission' and 'virulence' on the individual and social level.

Symposium - Monday, 12:15 9-STU

Disease profile of adult and juvenile edible crab (cancer pagurus) populations from the English channel fishery, UK.

Kelly Bateman - Cefas, Weymouth, Dorset, UK; ***Stentiford Grant*** - Cefas, Weymouth, Dorset, UK
Address for correspondence: kelly.bateman@cefas.co.uk

The edible crab (*Cancer pagurus*) is a major commercial species within Europe, supporting a large fishery. Despite their commercial value relatively little is known about their susceptibility to disease and the effect that these diseases may have upon populations. We carried out a disease survey on juvenile and adult edible crabs from a fished population within the English Channel. Using histology, molecular biology and electron

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microscopy we surveyed thirty juvenile and thirty adult crabs each month over a 12-month period. We found that the juvenile and adult sample groups displayed significantly different disease profiles (types of pathogen found) and prevalence of specific diseases. Both juvenile and adult crabs were susceptible to shell disease, *Hematodinium* sp., a microsporidian (*Enterospora canceri*) and a yeast-like organism. *Paramarteilia canceri* was found to be present within the adult population alone. In addition juvenile crabs were susceptible to a virus (Cancer pagurus Bacilliform Virus, CpBV), a novel Haplosporidian pathogen, a digenean (*Microphallus primas*), a turbellarian flatworm parasitoid (*Fecampia erythrocephala*), and a parasitic barnacle (*Sacculina carcini*). Of these pathogens exclusively found in juvenile crabs, the Haplosporidian, with monthly prevalence of up to 70% appeared to be the most significant, leading to hypertrophy and degeneration of the antennal gland and bladder of infected crabs. The study has highlighted important differences between diseases affecting juvenile and adult crab populations and further, the potential role of disease in leading to un-monitored ('silent') mortalities in the unfished juvenile stock. This presentation will describe the pathologies and prevalence of these diseases during the 12-month study.

CONTRIBUTED PAPERS — Monday, 10:30 - 12:30
White Pine I-II

Bacteria I

Moderator: Hyun-Woo Park

Contributed Paper - Monday, 10:30 — 10

Proteomic analysis of the crystal and spore mixture from six *Bt* strains to search for novel mosquitocidal proteins.

Yunjun Sun - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University, Panama City, Florida, USA; ***Hyun-Woo Park*** - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University, Panama City, Florida, USA
Address for correspondence: syj6253@163.com

Previously we described a rapid and accurate method for analyzing protoxin expression profile in *Bacillus thuringiensis* strains. In this method, solubilized protoxins are directly embedded into a polyacrylamide gel block without electrophoresis. After in-gel digestion of proteins, LC-MS/MS is used to analyze the extracted peptides for protein identification. Given its technical simplicity and sensitivity, we have adopted this experimental method in this study to search for novel mosquitocidal proteins from the six known *B. thuringiensis* strains. *B. thuringiensis* subsp. *galleriae* 4G5, subsp. *darmstadiensis* 4M1, subsp. *canadensis* 4H2, subsp. *fukuokaensis 4AP1, subsp. malayensis 4AV1 and subsp. jegathesan were selected, and their crystal-spore mixtures were analyzed accordingly. Among these strains, we detected Cry1, Cry2, and Cry9 protoxins in 4G5, Cry1 in 4M1, Cry9 in 4AP1, Cry15 in 4AV1, Cry11, Cry15, Cry24, Cry30, and Cyt2 in subsp. jegathesan, but no insecticidal protoxin was detected in 4H2. Besides these protoxins, other proteins not belonging to protoxins were also detected. The cognate genes encoding part of the detected proteins were confirmed by PCR analysis with general primers. This study not only revealed the protoxin expression profile in these B. thuringiensis strains, but also found one interesting mosquitocidal protoxin, Cry30, which has not been reported in the mosquitocidal B. thuringiensis subsp. jegathesan. Also, the results demonstrated that the total cry gene content of B. thuringiensis strains may be higher than previously thought. Finally, the non-protoxin proteins detected in this study provide us an opportunity to search for some other kinds of insecticidal proteins and possible functional proteins.*

Contributed Paper - Monday, 10:45 — 11-STU

Comparing the midgut epithelial regenerative response in *Bt*-susceptible and -resistant *Heliothis virescens* larvae.

Anaïs Castagnola - University of Tennessee, Knoxville, Knoxville, Tn, USA; ***Juan Luis Jurat-Fuentes*** - University of Tennesee, Knoxville, Knoxville, Tn, USA; ***Shigetoshi Eda*** - University of Tennesee, Knoxville, Knoxville, Tn, USA
Address for correspondence: atorrijo@utk.edu

Pluripotent stem cells in the midgut epithelium of *Heliothis virescens* larvae generate morphologically distinct daughter cells with a precise physiological function. We have developed a fluorescence-based method to discriminate and quantify midgut mature and stem cells in a preparation. Our current hypothesis is that stem cells replenish dying mature cells during gut intoxication by responding to factors secreted by dying cells. The goal of the present work was to determine differences in the gut regenerative process between susceptible and Bt-resistant *H. virescens* larvae. We report on the isolation of Cry toxin-induced secretomes from primary mature larval gut cell cultures from susceptible (YDK, COW) and resistant (CXC, YHD2, KCBhyb) *H. virescens* larvae. Even though stem cells from susceptible and resistant strains responded similarly to secretomes from a specific strain, secretomes from different strains induced distinct responses on the stem cells. Using our flow cytometry method we were able to quantify the differential proliferation and differentiation effect induced by factors in secretomes from each strain. Our results suggest a direct correlation between the diverse responses induced by secretomes and resistance to Cry toxins in *H. virescens*.

Contributed Paper - Monday, 11:00 — 13-STU

Lpss are virulence factors of the Mexican *Serratia entomophila mor4.1* to *Phyllophaga blanchardi* larvae (Coleoptera).

Zitlhally Rodriguez-Segura - Centro de Investigación en Biotecnología,2 Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico; ***Jean Chen*** - Depts. of Cell Biology and Neuroscience, Riverside, California, USA; ***Francisco Villalobos*** - Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico; ***Sarjeet Gill*** - Depts. of Cell Biology and Neuroscience, Riverside, California, USA; ***Maria Eugenia Nuñez-Valdez*** - Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico
Address for correspondence: zit_rodriguez@hotmail.com

Scarabs larvae of the *Phyllophaga* genus (Coleoptera:Scarabaeidae) are soil dwelling pests, feeding on plant roots causing damage to crops and grass lands. A Mexican strain of *S. entomophila* (SeMor4.1) pathogenic to *Phyllophaga* spp. by oral inoculation and to *Anomala* sp. and to the lepidopteran *Manduca sexta* by injection, has been isolated from the haemocoel of a dead larva. (Nuñez-Valdez et al., 2008. Appl. Environ. Microbiol. 74(3):802-10.). Toxin-like virulence factors are involved in pathogenicity. Insecticidal activity is caused by the bacteria or by cell free culture broths. To identify and characterize the virulence factors a genomic approach was followed by constructing a Se Mor4.1 fosmid library in *E. coli.* The library clones carrying virulence factors were screened by injecting individual clones into *Phyllophaga* sp. larvae and those able to kill the larvae were selected. The functional characterization of the clone named G8 was performed. Transposon insertion mutagenesis of G8 and analysis of the loss of insecticidal activity by injection bio-assays showed that a putative dUTPase, a Flavoprotein and a Glycosiltransferase-9, are involved in insecticidal activity. The latter protein is a component of the lipopolysaccharide (LPS) biosynthesis core, known as an enterobacterial

endotoxin with potent toxic activity by injection to invertebrates (Bennett and Clarke, 2005. J Bacteriol. 187:77-84.). Injection of the purified LPS from G8 has toxic activity to larvae of *P. blanchardi*, whereas the LPS from G8 mutants lost toxic activity. Similar bio-assays injecting cell free broths from clones carrying the dUTPase and Flavoprotein in one plasmid and the Glycosiltransferase-9 in other plasmid suggested that the toxic activities of the former proteins are independent from the latter. It is suggested that the whole insecticidal activity of G8 is linked to the LPS and other extracellular components, which could be released into outer membrane vesicles (OMVs) functioning as carriers of virulence factors.

Contributed Paper - Monday, 11:15 14-STU

Overproduction of Cry2ac by modification of nucleotide sequences between *rbs* and start codon in.

Faiza Saleem - School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan, Lahore, Punjab, Pakistan; ***Hyun-Woo Park*** - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center Florida A & M University, Panama City, Florida 32405, U.S.A., Panama City, Florida, USA; ***Muhammad Akhtar*** - School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan, Lahore, Punjab, Pakistan; ***Abdul Rauf Shakoori*** - School of Biological Sciences, University of the Punjab, Lahore 54590, Pakistan, Lahore, Punjab, Pakistan
Address for correspondence: zoologist1pk@yahoo.com

Insecticidal *Cry* toxins that *B. thuringiensis* produce during the sporulation have been employed for biological control of pest insects through formulations. Various strategies have been used to increase the yield of these toxins per unit medium. *Cry2Ac* has dual toxicity against certain lepidopterous and dipterous insects. The *cry2Ac* gene appears as a third gene in an operon that consists of three open reading frames (ORFS). However, crystallization of the *Cry2Ac* protein requires the second ORF (ORF2) in the *cry2Ac* operon as a scaffolding protein. In order to enhance activity of the *Cry2Ac toxin from HD29 strain of B. thuringiensis subsp. galleriae (Bacillus Genetic Stock Center ID 4G5; Serotype 5a5b), the gene was overexpressed in B. thuringiensis by genetic modification. The number and the sequence of the nucleotides between ribosome-binding sequence (RBS) and start codon (ATG) were modified and the genes with modified upstream sequences were cloned in pSTAB shuttle expression vector containing the cyt1A promoters from B. thuringiensis subsp. israelensis combined with the STAB-SD sequence from B. thuringiensis subsp. morrisoni strain tenebrionis. The resulting plasmids were introduced in 4Q7, an acrystalliferous mutant strain of B. thuringiensis subsp. israelensis and the Cry2Ac production of recombinant strains was confirmed by SDS-PAGE. Synthesis of Cry2Ac varied greatly throughout the recombinants with up to 10-fold increase compared with the wild-type Cry2Ac. Accordingly, crystals of the over-produced Cry2Ac were much larger than those of the wild-type.*

Contributed Paper - Monday, 11:30 15

High expression of pathogen-induced genes in a *Spodoptera exigua* colony resistant to *Bacillus thuringiensis*.

Patricia Hernández-Martínez - Department of Genetics, Universitat de València, Spain, Burjassot, Valencia, Spain; ***Gloria Navarro-Cerrillo*** - Department of Genetics, Universitat de València, Spain; ***Ruud A. de Maagd*** - Plant Research International B. V., The Netherlands; ***William Moar*** - Department of Entomology and Plant Pathology, Auburn University, USA; ***Juan Ferré*** - Department of Genetics, Universitat de València, Spain; ***Baltasar Escriche*** - Department of Genetics, Universitat de València, Spain; ***Salvador Herrero*** - Department of Genetics, Universitat de València, Spain
Address for correspondence: sherrero@uv.es

Products based on the entomopathogenic bacteria *Bacillus thuringiensis* are extensively used for the biological control of species of the orders Lepidoptera, Diptera, and Coleoptera. Differences in gene expression between susceptible and resistant colonies can reveal the resistance basis to these products. In this work, we have combined Suppression Subtractive Hybridization (SSH) libraries and DNA-macroarray to compare the midgut gene expression between two colonies of *Spodoptera exigua*, one of them highly resistant to a *B. thuringiensis* based commercial product. The results revealed a strong overexpression in the resistant colony of several genes that have been previously reported in response to pathogenic bacteria, viruses, or Cry toxin intoxication. DNA-macroarray values were validated by quantitative PCR confirming the overexpression these genes. Additionally, we have detected correlation between the expression of two of these genes and the resistance to *B. thuringiensis* in the analyzed colony.

Contributed Paper - Monday, 11:45 16

Cry1ac receptors and susceptibility during heliothine larval development.

Weibing Shi - Zhejiang University, Hangzhou, Zhejiang, P. R. China; ***Cris Oppert*** - University of Tennessee, Knoxville, TN, USA; ***Juan Luis Jurat-Fuentes*** - University of Tennessee, Knoxville, TN, USA
Address for correspondence: jurat@utk.edu

Correlations between larval susceptibility and Cry toxin binding during development have been previously studied. However, the specific levels of putative toxin receptor proteins and their correlation with toxin binding have not been reported. In this work, we tested correlations between toxicity to Cry1Ac, levels of proposed functional Cry1Ac receptors (cadherin, alkaline phosphatase and aminopeptidase-N), and Cry1Ac toxin binding in larvae of *Heliothis virescens* and *Helicoverpa zea*. Our data suggest that susceptibility, toxin binding, and receptor expression are not always correlated during heliothine larval development.

Contributed Paper - Monday, 12:00 17-STU

The development of the *Cry8*-type genes from *Bacillus thuringiensis* toxic to scarabs.

Guixin Yan - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China; ***Fuping Song*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China; ***Zhihong Lang*** - Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China; ***Dafang Huang*** - Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China; ***Jie Zhang*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China
Address for correspondence: jzhang@ippcaas.cn

Scarabs cause significant reduction in productivity and substantial economic loss. The *cry8*-type genes of *Bacillus thuringiensis* toxic to scaraboid larvae offer a practical alternative for the biocontrol of the pests. Until recently, there were sixteen *cry8* holo-type genes cloned in the Globe. In our lab, mainly four *B. thuringiensis* strains named HBF-1, HBF-18, Bt185 and BtSU4 toxic to Scarabs were revealed. Five holotype genes were cloned from these strains respectively. HBF-18 containing *cry8Ga1* was insecticidal to *Holotrichia oblita* and *Holotrichia parallela*. The strain Bt185 (containing *cry8Ea1* and *cry8Fa1*) and BtSU4 (containing *cry8Ha1* and *cry8Ia1*) were all toxic to *H. parallela*.

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The strain HBF-1 containing *cry8Ca2* showed activity against *Anomala corpulenta*. In addition to, the activated toxin of Cry8H by chymotrypsin showed high toxicity towards neonate larvae of one kind of leaf beetle --*Colaphellus bowringi* (Coleopteran). To make full use of the toxic strains and genes, the modified gene, transgenic microbe and turfgrass were studied. Mutant genes, M102 (E 642 G), and M102 (Q 439 P) were more toxic compared with Cry8Ca2. Two engineered strains, 3A-SU4 and 3A-HBF constructed by electroporation, were highly toxic not only to scarabaeidae pests, but also chrysomelidae; Engineered strain BIOT185 constructed by homologous recombination showed high toxicity to both *H. parallela* and *A. corpulenta*. Field experiments results showed the potential of biocontrol for different scarabs; *cry8Ea1* and *cry8Ga1* genes were transformedinto creeping bentgrass (*Agrostis stolonifera*) through *Agrobacterium umefaciens*, and transgenic creeping bentgrass (*A .stolonifera*) resistant to insect was gained successfully.

Symposium (Cross Divisional) Monday, 14:00 - 16:10
Kokopelli Ballroom II

Epizootiology and Its Impact on Microbial Control—Honoring the Work of Jim Fuxa

Moderator: David Shapiro-Ilan
Organizers: David Shapiro-Ilan, Ann Hajek

Symposium - Monday, 14:00 18

Epizootiology of fungi.

Ann E. Hajek - Department of Entomology, Ithaca, New York, USA
Address for correspondence: aeh4@cornell.edu

Factors influencing the prevalence of fungal diseases in invertebrate populations can be viewed in the context of the fungal pathogen, the invertebrate host and the surrounding biotic and abiotic environment, as well as interactions among these factors. The combinations of conditions that result in disease epizootics are unique to different host/pathogen systems, few of which have received much study. In this presentation, aspects of the epizootiology of fungal diseases that have received more study will be discussed, especially with regard to use of this information toward development of microbial control. Methods are constantly improving for quantifying pathogen propagules in the environment as well as quantifying the prevalence of disease in host populations. When and where most hosts get infected is critically important toward being able to predict the occurrence of epizootics, a goal for microbial control. Likewise, factors linked with survival of pathogen propagules under different conditions are important to understand. Pathogenicity and virulence of fungal strains strongly affect levels of infection. Behavioral responses of hosts to pathogen propagules as well as behavioral changes of infected hosts are known to impact the course of infections. Studies of interactions of pathogens and diseased hosts in biotic communities are relatively few, although plants used by herbivorous invertebrates as well as interactions with other natural enemies are known to influence fungal pathogens. Models have been developed to understand disease dynamics through space and time and spread and localized dynamics of entomopathogenic fungi are presently being studied with the goal of investigating metapopulation dynamics.

Symposium - Monday, 14:30 19

Epizootiology of nematodes.

Harry K. Kaya - University of California, Department of Nematology, Davis, CA, USA
Address for correspondence: hkkaya@ucdavis.edu

Infections caused by species in various nematode families are common in insects. In spite of their commonality in insect populations, epizootiological studies of nematode infections are few. The reasons for the few long-term studies include the following. Nematodes do not cause dramatic declines in insect populations, and their infections are not obvious in insect populations because they often do not kill their insect hosts. They can reduce fecundity or longevity, change their behavior, cause morphological aberrations, etc. Sometimes the prevalence of infection remains in an enzootic state suggesting that epizootics rarely take place. Many nematodes and their hosts occur in cryptic habitats (e.g., soil) and it is difficult to follow the nematode and host populations over a long term. Such factors may reduce the appeal for long-term studies dealing with epizootiology of nematode infections. Thus, there are a numerous reports on prevalence of nematode infections in insect populations for the short term. However, there are some long-term studies, especially with nematodes that have been used in classical biological control programs. The best examples are with the insect host, *Sirex noctilio* (woodwasp), and the nematode, *Deladenus (Beddingia) siricidola* and with the insect hosts, *Scapteriscus* species (mole crickets) and the entomopathogenic nematode, *Steinernema scapterisci*. In addition, the use of other entomopathogenic nematode species (i.e., *Steinernema* and *Heterorhabditis*) for biological control of insect pests has spurred the examination of many factors that deal with epizootiological principles. These principles include the host population (i.e., density and stage), pathogen population (i.e., virulence and infectivity, persistence, and dispersal), transmission, and environmental factors. How these principles affect epizootiology of nematode diseases will be explored.

Symposium - Monday, 15:00 20

Epizootiology of microsporidia.

David Oi - USDA-ARS Center for Medical, Agricultural, & Veterinary Entomology, Gainesville, Florida, USA
Address for correspondence: david.oi@ars.usda.gov

Microsporidia are eukaryotic, obligate, intracellular parasites that in general, slowly debilitate their hosts. Epizootiology of microsporidia that infect insects has been generated much interest in the context of microbial control and integrated pest management. In addition, microsporidian epizootics in insectaries and laboratory cultures make understanding the factors and causes of diseases essential to maintaining operations. In tribute to Jim Fuxa, studies of microsporidian-insect systems that illustrate concepts of epizootiology that he articulated will be reviewed. Of particular emphasis will be the interaction of the microsporidian fire ant pathogen, *Kneallhazia (=Thelohania) solenopsae*, and the social form of its host. In this system, Jim documented the existence of relatively rare infections in populations of the single-queen social form of fire ants. It has since spurred theories on how this pathogen may spread relative to dispersal nuances of the host, and ideas for improving the pathogen's impact as a microbial biological control for this invasive ant.

Symposium - Monday, 15:30 21

Nucleopolyhedrovirus epizootiology: One researcher's perspective.

James Fuxa - Retired Professor, Baton Rouge, LA, USA
Address for correspondence: jimfuxa@yahoo.com

Highlights and hypotheses from 25 years of research in nucleopolyhedrovirus (NPV) epizootiology will be presented. Emphasis will be placed on realized heritability of host resistance, the influence of virulence on epizootics, vertical transmission as a transport and possibly a resistance phenomenon, biotic and abiotic NPV transport, viral persistence in soil and in the ecosystem, threshold concentrations of NPV in soil to initiate epizootics, permissiveness of ecosystems in epizootics, epizootiology in microbial control including classical biocontrol in an ephemeral row crop, and epizootiology in risk assessment. Examples will be drawn from fall armyworm (*Spodoptera frugiperda*) in pastures and corn, velvetbean caterpillar (*Anticarsia gemmatalis*) and soybean looper (*Pseudoplusia includens*) in soybean, *A. gemmatalis* in wild marsh legumes, and DNA-recombinant viruses with tobacco budworm (*Heliothis virescens*) and cabbage looper (*Trichoplusia ni*) in cotton and collards microcosms.

Symposium - 16:00 22

Panel Discussion, Summary.

David Shapiro-Ilan - USDA-ARS, Byron, Georgia, USA
Address for correspondence: david.shapiro@ars.usda.gov

CONTRIBUTED PAPERS Monday, 14:00 - 16:00
White Pine I-II

Invertebrate Immunity

Contributed Paper - Monday, 14:00 23-STU

Consumption of *Bacillus thuringiensis* and selection for *Bt*resistance is associated with changes in insect immune function.

Jerry Ericsson - Simon Fraser University, Burnaby, British Columbia, Canada; ***Judith Myers*** - University of British Columbia, Vancouver, British Columbia, Canada; ***Carl Lowenberger*** - Simon Fraser University, Burnaby, British Columbia, Canada
Address for correspondence: jericsso@sfu.ca

The innate immune system of *Trichoplusia ni* has the potential to mitigate the lethal effects of *Bacillus thuringiensis kurstaki* (Btk) spore-crystal formulations. We studied the immune response to Btk in susceptible and resistant *T. ni* after consumption of sub-lethal concentrations of the sporecrystal formulation, and quantified the expression of genes coding for antimicrobial peptides (AMP), and the differential number of circulating hemocytes in resistant and susceptible individuals. AMP expression levels in fat bodies were compared after an injection challenge with a cocktail of Gram negative *(Escherichia coli)* and Gram positive *(Staphylococcus epidermidus)* bacteria, and after consumption of Btk. We found that the resistant (BTR) *T. ni* could tolerate Btk concentrations >200-fold higher than susceptible (BTS) lines, and that a standardized microbial injection caused a significant increase in the number of circulating hemocytes in BTS but not in BTR. Consumption of Btk caused significant reductions in the number of hemocytes in BTS, but not BTR. Significant differences in transcript levels of genes coding for defensin A, lysozyme A, and gloverin were found by quantitative real-time PCR, and colony, time since exposure, and treatment all had a significant effect. The overall changes in cellular and humoral immune factors after consumption of Btk suggest that multiple systems are stimulated, and together may contribute to reducing the toxic effects of Btk.

Contributed Paper - Monday, 14:15 24-STU

Upregulation of circulating hemocytes in response to bacterial challenge is mediated by biogenic amines via *rac1* signal.

Geun Seob Kim - Andong National University, Andong, Gyeong Buk, Korea; ***Yonggyun Kim*** - Andong National University, Andong, Gyeong Buk, Korea
Address for correspondence: ttogye@naver.com

Octopamine and 5-hydroxytryptamine (5-HT) have been known to mediate insect immune responses. This study investigated a mediating role of both monoamines in the change of circulating hemocyte populations due to bacterial challenge in the beet armyworm, *Spodoptera exigua*. To analyze the enhanced mobilization of hemocytes into a circulating form, we tested a functional interaction of both monoamines signals with Rac1 that is a member of the subfamily of the Rho family of GTPases, which induce cell growth, cell-cell adhesion, motility, and cytoskeletal activation. Bacterial challenge induced significant increase of total hemocyte populations in hemolymph within 4h. This increase in the circulating hemocyte counts was elicited by injection of either octopamine or 5-HT without any bacterial challenge. On the other hand, their specific antagonists, phentolamine (an octopamine antagonist) or ketanserin (a 5-HT antagonist) suppressed the increase of the circulating hemocyte counts in response to bacterial challenge. The rapid increase of the circulating hemocyte counts was not explained by de novo hemocyte production from hematopoietic organ, but appeared to come from a sessile hemocyte reservoir because a treatment of ligation between thorax and abdomen did not inhibit the increase of hemocyte counts in the isolated abdomen in response to bacterial challenge. These mediatory activities of both monoamines were not dependent on eicosanoids due to no effect of dexamethasone on their hemocyte recruitment. An adenylate cyclase inhibitor, NKY80, significantly impaired the hemocyte mobilization. Also, Rac1 inhibitor significantly antagonized the monoamine effects to increase circulating hemocyte populations. Rac1 activity was necessary to change hemocyte shape, which was quantitatively analyzed with hemocyte-spreading behavior. This study suggests that octopamine and 5-HT mediate a rapid increase of circulating hemocyte populations via Rac1 signal in *S. exigua*.

Contributed Paper - Monday, 14:30 25-STU

withdrawn

Contributed Paper - Monday, 14:30 26

Transcriptome analysis of pathogen exposed *Spodoptera exigua* larvae.

Salvador Herrero - Department of Genetics. Universitat de Valencia, Burjassot, Valencia, Spain; ***Heiko Vogel*** - Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany
Address for correspondence: sherrero@uv.es

Insect response to pathogens is mainly mediated by changes in their transcriptional profile. Nowadays, transcriptional information available for model organisms is relatively large although it is still very limited for non-model organisms such as the Lepidoptera *Spodoptera exigua*. We have combined classical Sanger sequencing with novel 454-based pyrosequencing in order to increase the information available about the larval transcriptome of this insect. In total, around 20.000 ESTs with

an average length of 800 bp were obtained from different tissues of larvae exposed to different pathogens. Studied tissues include: midgut, fatbody, hemocytes and integument of fourth instar larvae. These tissues were obtained from larvae exposed to pathogens such as gram+ and grambacteria, yeast, baculovirus, and also to *Bacillus thuringiensis* toxins. Annotation of these ESTs has revealed the presence of several members of the main signaling pathways involved in the insect response to pathogens. Moreover, sequence analysis has also allowed the identification of novel viral species present in the larvae of this insect. Obtained data will be summarized and their possible applications will be discussed.

Contributed Paper - Monday, 14:45 27

Detection and analysis of innate immune genes of the termite.

Drion Boucias - University of Florida, Gainesville, FL, USA; ***John Denton*** - University of Florida, Gainesville, FL, Alachua; ***Tamer Salem*** - University of Florida, Gainesville, FL, USA; ***Michael Scharf*** - University of Florida, Gainesville, FL, USA
Address for correspondence: pathos@ufl.edu

The subterranean termite, lives in a moist, protected environment that is well-suited for microbial development. Colony members are in constant contact, thus increasing the likelihood of disease transmission. In nature, epizootics are uncommon and few entomopathogens have been detected infecting this eusocial insect. In part, disease resistance is due to social behaviors responsible for pathogen removal and disruption of these behaviors leads to a dramatic increase in disease incidence. An important characteristic of R. flavipes is the presence of commensalistic microbiota; how these commensals survive, multiply, and cycle through termites without triggering an antimicrobial response is unknown. As an initial step in understanding the response of the gut tissue to microbial elicitors we are investigating the innate immune transcripts. Forty putative genes associated with the innate immune system have been identified in our gut cDNA library. These include: PRPs (peptidoglycan recognition proteins, gram-negative bacterial-binding proteins and lectins) transcription components (CJun1); immune signaling molecules (ankyrins, SOCS box-containing protein, leukotriene A-4 hydrolase, and tetraspanin); apoptosis-related genes (programmed cell death protein, autophagyrelated proteins, and apoptosis inhibitory proteins); AMPs (defensin precursors, cationic peptide CP8 precursor, glycine-rich peptides, salivary cysteine-rich peptides); protease inhibitors (iron metabolism genes, transferrin and ferritin); and various enzymes (SODs, kinases, serine proteases, laccase, peroxidases, catalase, lysozymes, and prophenoloxidase-activating factors.) A combination of 3' and 5' RACE reactions was conducted to provide complete sequence data of open reading frames including the untranslated regions. Details of the functional epitopes and phylogenies of selected innate proteins will be presented.

CONTRIBUTED PAPERS Monday, 14:00 - 16:00
Painted Horse I-II

Nematodes I

Moderator: Kelly Sims

Contributed Paper - Monday, 14:00 28

The screening symbiotic bacteria and its purification and gene clone of insecticidal proteins from *Xenorhabdus poinarii SY5.*

Bin Cong - Laboratory for Bio-control,Shenyang Agricultural University, Shenyang City, Liaoning, China; ***Huan Wang*** - Laboratory for Bio-control, Shenyang Agricultural University, Shenyang, Liaoning, China; ***Xihua Wang*** - Laboratory for Bio-control, Shenyang Agricultural University, Shenyang, Liaoning, China; ***Hui Dong*** - Laboratory for Bio-control, Shenyang Agricultural University, Shenyang, Liaoning, China; ***Haitao Qian*** - Laboratory for Bio-control, Shenyang Agricultural University, Shenyang, Liaoning, China
Address for correspondence: bin1956@163.com

152 strains of symbiotic bacteria were isolated from 28 strains of entomopathogenic nematodes .The bioassay indicated the all bacteria strains had oral insecticidal activity. The insecticidal toxins of the 28 highly virulent strains were screened from them. Among them the SY5 stain of *X. poinarii*, isolated from Shenyang city, China, showed both insecticidal and antibiotic activities. Highly insecticidal virulence to Galleria mellonella Ostrinia furnacalis Plutella xylostella Mythimna separata *Laphygma exigua* and *Tenebrio molitor* and highly antibiotic activities to *Colletotrichum lindemuthianum, Colletotrichum truncatum, Fusarium wilt, Fusarium wilt, Curbularia lunata, Bipolaris maydis, Sporisorium reilianum, Fusarium species, Pyricularia oryzae, Botrytis cinerea, Alternaria solani, Cercospora fuligenae*Roldan, *Fulvia fulva, Gibberella zeae, Bipolaris sorokiniana, Sclerospora graminicola, Alternaria alternate* Keissler, *Cordana musae* Hohn, *Fusaritun*, *Colletotrichum gloeosporiodies*. A purification procedure was applied to isolating the toxin of *X. poinarii* SY5, using precipitation with ammonium sulfate and DEAE-52 chromatography. Insecticidal activities of the extracellular secretions were examined by oral bioassay in purified clone. Seven toxins were purified. The insecticidal activity of toxin, toxin and toxin were higher than other toxins. The genome cosmid library was constructed in order to clone insecticidal genes from *X. pionarii* SY5 strain. The two clones with oral activity against neonate *O. furnacalis* larvae were obtained after screening 420 *E. coli* clones by the bioassay. The two clones with oral activity against neonate *O. furnacalis* larvae were obtained and given the numbers Supercos 315 and Supercos 367. 17 pairs of primers were designed according to the insecticidal toxin genes of *X. nematophilus* PMFI296 in GenBank to PCR amplification of toxin gene fragments. One fragments were obtained from Supercos 315 and Supercos 367 respectively. Online BLAST analysis showed that the sequences of Supercos 367 PCR products had 84% homology to the toxin genes of *X. nematophilus* PMFI296.

Contributed Paper - Monday, 14:15 29

The *Wolbachia* endosymbiont as a potential anti-filarial nematode target.

Bo Wu - Molecular Parasitology Division, Ipswich, MA, USA; ***Jacopo Novelli*** - Molecular Parasitology Division, Ipswich, MA, USA; ***Jeremy Foster*** - Molecular Parasitology Division, Ipswich, MA, USA; ***Barton Slatko*** - Molecular Parasitology Division, Ipswich, MA, UAS
Address for correspondence: slatko@neb.com

Over 1 billion people in more than 90 countries are at risk from filarial nematode infections, with 150 million people infected. The parasitic nematodes are insect-borne and are responsible for lymphatic (elephantiasis) or cutaneous filariasis (Onchocerciasis/African River Blindness). Within these filarial parasites are obligate intracellular alphaproteobacteria, *Wolbachia*. Current anti-filarial chemotherapy can interrupt transmission by killing larvae, but is less effective on adult worms, which can live 10-15 years in humans. There is an urgent need to develop adulticidal drugs. Over the last several years, the obligate endosymbiont *Wolbachia* has been recognized as a potential target for filarial nematode life cycle intervention as evidenced by the loss of worm

fertility and viability upon antibiotic treatment, including in human trials. However, current drug treatments are not practical due to the dosages and length of required treatments. Nevertheless, anti-*Wolbachia* targeting appears promising for filariasis control. The symbiotic relationship between *Wolbachia* and its nematode host remains elusive. Comparative genomics and bioinfomatic analysis has identified a number of potential interactions which may be drug targets, of which one is de novo heme biosynthesis, due to its absence in the host nematode *Brugia malayi* genome sequence but presence in the *Wolbachia* genome sequence. We will describe our worm viability assays which suggest that both female and male *B. malayi* adult worms are killed by heme biosynthesis-specific inhibitors. We will also describe our cloning, over-expression and analysis of the enzymes of the heme biosynthetic pathway for preparing proteins for drug targeting and our development of an *E. coli* functional complementation drug targeting strategy. Finally, we will describe our approaches for understanding the symbiotic relationship and identifying potential drug targets by analysis of lateral gene transfer events and initial proteinprotein interaction studies.

Contributed Paper - Monday, 14:30 30-STU

Mutational analysis yields insight into the function of *nilb,* a specificity determinant in an animal-bacteria symbiosis.

John Chaston - Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA; ***Heidi Goodrich-Blair*** - Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA
Address for correspondence: chaston@wisc.edu

The monospecific association between *Steinernema carpocapsae* (Nematoda) and the enterobacteria *Xenorhabdus nematophila* is a model for studying animal-microbe associations and animal-animal parasitism. Together the pair parasitize soil-dwelling insects, predominantly lepidopterans. Although the nematode is able to kill the insect host in the absence of its symbiotic bacteria, the bacteria play an integral role in killing the insect when present and are essential for nematode reproduction. Effective carriage of the bacteria between insects, which occurs within a specialized portion of the intestine of the infective stage nematode, is therefore crucial for nematode reproductive fitness. One bacterial factor that is essential for nematode colonization is the outer membrane protein NilB (nematode intestine localization). To identify regions of NilB that play a role in nematode colonization we created in-frame deletions of several predicted extracellular and periplasmic domains and assessed the role of each in the process of nematode colonization. Our analysis revealed that different portions of NilB are essential for initiating nematode colonization, and for bacterial outgrowth and persistence within the nematode intestine. This work sheds light on the process of colonization and the role of NilB.

Contributed Paper - Monday, 14:45 31-STU

Transcriptional profiling of trait deterioration in the insect pathogenic nematode *Heterorhabditis bacteriophora.*

Bishwo Adhikari - Department of Biology, Brigham Young University, Provo, UT, USA; ***Chin-Yo Lin*** - Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT, USA; ***Parwinder Grewal*** - Department of Entomology, The Oho State University-OARDC, Wooster, OH, USA; ***David Shapiro-Ilan*** - USDA-ARS, Southeastern Fruit and Nut Research Laboratory, Byron, GA, USA; ***Byron Adams*** - Department of Biology, Brigham Young University, Provo, UT, USA
Address for correspondence: adhikaribn@byu.net

The success of a biological control agent depends on key traits, particularly compatibility with the target pest, reproductive potential, host-finding ability, environmental tolerance, and ability to be cultured. These traits, however, can deteriorate rapidly when a biological control agent is isolated from nature and mass cultured for commercial purposes. Entomopathogenic nematodes (EPNs) are widely used as bio-control agents and are exceptional models for the study of parasitism, pathogenicity, and symbiosis. Like other bio-control agents, traits can deteriorate rapidly in EPNs and their symbiotic bacteria. The physiological and biochemical mechanisms of trait deterioration in EPNs are well studied but the molecular mechanisms behind these genetic processes remain unclear. We studied the molecular mechanisms of trait deterioration of two experimental lines of *Heterorhabditis bacteriophora* that differed in their virulence, heat tolerance and fecundity. Microarray gene expression analysis of an inbred line (L5M) and its original parental line (OHB) showed that a large number of genes from different functional groups are differentially expressed (DE) in trait-deteriorated nematodes. Many of those DE genes are involved in metabolism while others were involved in signal transduction, transportation, translation, immune response and cellular processes. We observed the coordinated expression of a large spectrum of genes and huge changes in metabolic processing, indicating a clear shift from primary to secondary metabolic processes. The DE genes also included a number of potential molecules secreted or excreted in the host-parasite interaction that could play vital roles in insect parasitism and suppression of host defense mechanisms. The transcriptional profiling of these DE genes will be pursued by functional analyses methods. The analyses performed in our study may provide a better understanding of interspecies interactions of IPNs (e.g. parasitism, mutualism, and vector-borne disease) and will enhance the understanding of the mechanisms underlying trait deterioration in biological control agents.

Contributed Paper - Monday, 15:00 32

Genetic breeding for heat tolerance of *Heterorhabitis bacteriophora.*

John Mukuka - Kiel University, Kiel, Schlewig Holstein, Germany; ***Olaf Strauch*** - Kiel University, Kiel, Schlewig Holstein, Germany; ***Ralf Udo Ehlers*** - Kiel University, Kiel, Schlewig Holstein, Germany
Address for correspondence: ohn.mukuka@biotec.uni-kiel.de

High temperatures of over 30°C can occur during transportation of entomopathogenic nematodes to the end-user and this can result in loss of viability and quality of nematode products. The increase of heat tolerance by *H. bacteriophora* through genetic breeding is feasible as high heritability and response to selection of heat tolerance has been demonstrated already. This study investigated the possibilities to improve heat tolerance of the nematode *Heterorhabditis bacteriophora.* The study screened sixty *H. bacteriophora* strains on their survival at high temperatures and subsequently crossed the most heat tolerant strains to further improve heat tolerance. Prior to genetic crossings of the most tolerance strains, dauer juveniles from heat treatment that gave 5-10% survival (about 1000 individuals) were used for production of new progeny in the last instar *G. mellonella.* This presented two selection rounds before obtaining F2 generation of the hybrid strain. There were significant differences in heat tolerance among the *H. bacteriophora* strains. Adaptation to higher temperature for 3 h could not increase heat tolerance and no correlation was recorded for tolerance with or without adaptation. The mean tolerated temperatures ranged from 33.3°C to 40.1°C for non-adapted nematode populations and from 34.8° C to 39.2°C for adapted populations. The mean heat tolerance of hybrid strains was 40.3°C for non-adapted and 39.9°C after adaptation. These results confirmed superior performance of

hybrid strains over parental nematodes.

Contributed Paper - Monday, 15:15 33-STU

A model system for the identification of 'insects parasitism genes' and the investigation of 'parasitism gene' evolution.

Scott Peat - Department of Biology, Brigham Young University, Provo, UT, USA; ***Byron Adams*** - Department of Biology, Brigham Young University, Provo, UT, USA
Address for correspondence: speat@byu.net

Recent work in the field of pest management has focused on identifying genes involved in the parasitism of plants and then engineering the plants to disrupt (via RNAi) these parasitism genes as a means of controlling the plant pest. While numerous 'plant parasitism genes' have been identified in plant parasitic nematodes, no genes have yet been identified which are required for certain nematodes to parasitize insects. Furthermore, while the evolution of the parasitic lifestyle in nematodes has been addressed in numerous studies, few data exist that address the origin of parasitism and how it is maintained within the order Tylenchida. *Deladenus siricidicola*, a parasite of the woodwasp *Sirex noctilio*, provides an ideal system for both insect parasitism and plant parasitism gene exploration due to its phylogenetic position within the order Tylenchida as well as its two autonomous and trophically diverse life stages; a mycetophagus (fungal feeding) life stage and an entomophagus (insect parasitic) life stage. As such, we utilized expressed sequence tag (EST) data from *Deladenus siricidicola* to facilitate discovery of genes involved in insect parasitism and to investigate the evolution of plant parasitism genes. Expressed sequence tag data were generated by extracting total RNA, amplifying cDNA, and sequencing cloned cDNA. The resulting sequences were explored to identify potential insect parasitism and plant parasitism genes using sequence similarity, gene ontology assignment, and software that identifies orthologous genes across numerous EST datasets. Finally, tests of relative rates and selection were conducted to investigate gene evolution and selection in these 'parasitism genes'. This work establishes a framework for the exploration of parasitism genes and parasitism gene evolution, and provides valuable knowledge of parasitism gene evolution to those using these types of genes to engineer resistance to plant pathogens.

Contributed Paper - Monday, 15:30 34-STU

Evaluating the cost of environmental RNAi resistance in *Caenorhabditis elegans.*

Stephen Jenkins - Department of Biology, Brigham Young University, Provo, UT, USA; ***Barry Pittendrigh*** - Department of Entomology, University of Illinois at Urbana- Champaign, Urbana, IL, USA; ***David Onstad*** - Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA; ***Byron Adams*** - Department of Biology, Brigham Young University, Provo, UT, USA
Address for correspondence: sveninukraine@gmail.com

After the discovery of RNAi gene silencing in 1998, many scientists have recognized the potential of sequence-specific gene silencing as a method for conferring pest resistance to plants. Accordingly, plants can be genetically modified to contain specific dsRNA, which, when ingested, silences target genes in plant pests. Such strategies are said to be robust to the evolution of resistance because of the number of genes in the plant pests that serve as RNAi targets. To the contrary, we argue that pest populations could rapidly evolve resistance to RNAi targets through mutations in genes that code for proteins required by the RNAi pathways of plant pests. We selected 12 strains of *C. elegans* that are deficient in the *rde-1*, *rde-2*, *rde-4*, *rsd-2*, *rsd-3*, *rsd -4*, *rsd-6*, or *rsd-8* genes, conferring resistance to environmentally induced RNAi. By comparing their overall fitness to the wildtype strain (N2), we have shown costs associated with the evolution of resistance, manifest primarily in decreased fecundity. These results, combined with a better understanding of their molecular genetics, can be used to model the evolution of environmental RNAi resistance, and inform strategies of managing RNAi-based approaches to plant health.

Monday, 16:30 - 17:30
Kokopelli Ballroom I

POSTERS 1

Posters should be displayed from Monday 8:00 to Tuesday 17:30.

BACTERIA

Poster/Bacteria - Monday, 16:30 - 17:30 B-1-STU

***Manduca sexta* aminopetidase n and alkaline phosphatase are involved in binding with the oligomeric structure of Cry1ab toxin.**

Ivan Arenas - Instituo de biotecnología, UNAM., Cuernavaca, Morelos, México; ***Alejandra Bravo*** - Instituto de bitecnología, UNAM., Cuernavaca, Morelos, México; ***Mario Soberón*** - Instituto de biotecnología UNAM, Cuernavaca, Morelos, México; ***Isabel Gómez*** - Instituto de biotecnología, UNAM., Cuernavaca, Morelos, México
Address for correspondence: ivan@ibt.unam.mx

Bacillus thuringiensis is well know for its ability to produce parasporal crystalline protein inclusions (usually referred to as crystals), which have attracted worldwide interest for various pest management applications because of their pesticidal activity. The major threat to the use of the use of Cry toxins is the appearance of insect resistance and the most frequent mechanism of resistance to Cry toxins are defects in the binding with the receptors in the midgut of insects. The receptors reported for Cry1Ab toxin in lepidopteran insects include to a caderin like protein (Bt-R1), a GPI anchored proteins: aminopeptidase N (APN) and alkaline phosphatase (ALP), and a glycoconjugate of high molecular weight. It has been proposed that GPI anchored proteins interact with toxin oligomers that are produced after binding of the toxin monomer with Bt-R1. In this sense we are interested in understanding the role of GPI-anchored protein receptors in the interaction with the oligomer structure of the toxin. In this work we characterize the interaction of oligomer of Cry1Ab with ALP and APN. These receptors were purified from *Manduca sexta* midgut and we analyzed the interaction with oligomeric and monomeric toxin. The Cry1Ab oligomer binds with high affinity with ALP and APN, but the monomer binds with low affinity. A set of Cry1Ab mutants located in domain II and domain III were evaluated after oligomerization to describe the toxin regions involved in the interaction with each receptor.

Poster/Bacteria - Monday, 16:30 - 17:30 B-2

The effects of *Bacillus thuringiensis* on adult emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae).

Leah S. Bauer - USDA Forest Service, Northern Research Station, E. Lansing, MI, US; ***Diana Londono*** - Michigan State University, E. Lansing, MI, US; ***Libs John*** - Pyllom LLC, Mountain View, CA, USA
Address for correspondence: lbauer@fs.fed.us

The emerald ash borer (EAB) is a buprestid native to Asia, which is killing the ash trees (*Fraxinus* spp.) as it spreads throughout North America. Discovered in Michigan and Ontario in 2002, infestations of EAB are now known in areas of Indiana, Illinois, Maryland, Missouri, Ohio,

Pennsylvania, Virginia, West Virginia, and Wisconsin. EAB has killed tens of millions of ash trees since its arrival in infested solid-wood packing materials in the early 1990's. We are working towards development of a *Bacillus thuringiensis* (Bt) based microbial insecticide targeting adult EAB using aerial sprays. Although the phloem-feeding larvae of EAB are generally protected from topical sprays, EAB adults feed in the forest canopy on ash leaves. We screened over 20 coleopteran-active Bt strains for toxicity against EAB adults and found Cry8Da produced by Bt SDS-502 is highly toxic to EAB adults. Using electron microscopy, we found the effects of this Bt on EAB adult midguts are similar to those reported for other insects intoxicated with Bt. Using laboratory bioassays, we also found this Bt strain was not toxic to the parasitic hymenopterans being released for biological control of EAB. We will discuss the results of laboratory and field trials with formulated product.

Poster/Bacteria - Monday, 16:30 - 17:30 B-3

Characterization of Cry1ac binding and pore formation in Cry1ac-resistant *Helicoverpa zea* (Boddie).

Silvia Caccia - University of Valencia, Burjassot (Valencia), Valencia, Spain; ***Jayadevi Chandrashekhar*** - Auburn University, Auburn, Alabama, USA; ***William J Moar*** - Auburn University, Auburn, Alabama, USA; ***Juan Ferré*** - University of Valencia, Burjassot (Valencia), Spain
Address for correspondence: silvia.caccia@uv.es

Due to the increase in the adoption of crops expressing *Bacillus thuringiensis* insecticidal proteins, it is pivotal to understand the mechanisms by which the target pests could develop resistance. In a previous work with a laboratory-selected *Helicoverpa zea* colony (AR) resistant to Cry1Ac toxin it was shown that resistance was not linked to an alteration in binding, which is the most common feature associated to high levels of resistance. To prevent the elimination of AR due to fitness costs, AR was outcrossed with another laboratory colony that had been infused with insects collected from corn fields, and reselected for resistance to Cry1Ac toxin using an F2 screen. The resulting AR1 is more than 200-fold resistant to the toxin, almost twice as resistant as the original AR colony. Alteration in binding has been shown not to be the primary mechanism of resistance in AR. In the present study binding analyses were conducted with AR1 to determine whether the higher resistance level could reveal an alteration in the binding or in the pore formation capacity of Cry1Ac. Additionally, the crossing of AR with another susceptible colony could have resulted in the introduction of other Bt resistance alleles (such as altered binding) being transferred to AR. The results have shown that AR1 insects bind Cry1Ac with slightly less affinity (two-fold) than the susceptible insects. However, there were no differences at the level of concentration of binding sites, percentage of irreversible binding, or pore formation properties between both strains. Thus, as in the original AR colony, alteration of binding is not the major mechanism of resistance of the new Cry1Ac-selected cotton bollworm strain, though it might contribute to resistance along with other mechanisms such as alterated midgut proteolysis, as suggested in the literature.

Poster/Bacteria - Monday, 16:30 - 17:30 B-4-STU

Silencing of *Manduca sexta* aminopeptidase n1 by feeding double stranded RNA to study the receptor function *in vivo*.

Biviana Flores-Escobar - Instituto de Biotecnología UNAM, Cuernavaca, Morelos, México; ***Alejandra Bravo*** - Instituto de Biotecnología UNAM, Cuernavaca, Morelos, México; ***Mario Soberón*** - Instituto de Biotecnología UNAM, Cuernavaca, Morelos, México; ***Isabel Gómez*** - Instituto de Biotecnología UNAM, Cuernavaca, Morelos, México
Address for correspondence: biviana@ibt.unam.mx

The Cry toxins, produced by *Bacillus thuringiensis* (Bt), have an outstanding interest because of their specific insecticidal activity. Their use to control pest in forest and crops is regarded as environmentally friendly; however, there is still a risk in the employment of these toxins in the field: the emerging resistance of insects. Understanding the mechanism of action of Cry toxins is a major goal to counteract resistance and the most frequent appearance of resistance is the deficient interactions of receptors with the toxins. At least four different proteins that have a receptor activity for lepidopteran-specific Cry1A toxins have been described: a cadherin like receptor, an aminopeptidase–N anchored by glycosylphosphatidyl inositol (GPI), a GPI – anchored alkaline phosphatase, and a 270 kDa glycoconjugate. In this work, we focused on the study of the in vivo receptor function of *Manduca sexta* aminopeptidase N1 and its interaction with the Cry1Ab toxin. In order to accomplish our objective, we fed 1st instar *Manduca sexta* larvae with dsRNA corresponding to 3 different regions of the aminopeptidase N1 and then expose these silenced larvae to the Cry1Ab toxin. Our results show that the production of APN-1 is greatly reduced in silenced larvae. We will present data on the effect of the Cry1Ab toxin on silenced larvae and discuss the role of this receptor in the mode of action of Cry1Ab toxin.

Poster/Bacteria - Monday, 16:30 - 17:30 B-5

Loop residues of the receptor binding domain of *Bacillus thuringiensis* Cry11ba toxin are important for mosquitocidal activity.

Supaporn Likitvivatanavong - UCR, Riverside, CA, USA; ***Karlygash Aimanova*** - UCR, Riverside, CA, USA; ***Sarjeet Gill*** - UCR, Riverside, CA, USA
Address for correspondence: spplikit@gmail.com

Cry11Ba toxin is one of the most toxic single *Bacillus* toxin to mosquito larvae. It has been proposed that the mechanism of the *Bacillus thuringiensis* Cry protein action involves sequential receptor bindings, membrane penetration and pore formation. The binding to a receptor is a crucial first step, and specific toxin domains are involved in this binding. To identify these specific domains we first developed a model of the Cry11Ba toxin and identified predicted loop regions in domain II. Four peptides derived from loop alpha 8, loop 1, loop 2 and loop 3 were tested for binding assays on *Aedes* BBMV. Three peptides corresponding to alpha 8, loop 1 and loop 3 competed with binding of the activated-Cry11Ba toxin. The Cry11Ba toxin binds to the apical membrane of caeca and posterior midgut of *Aedes* larvae. This binding can be competed by the loop 1 peptide peptide, providing further evidence of the importance of loop 1 in receptor binding. These same loop regions are also involved in binding cadherin and alkaline phosphatase.

Poster/Bacteria - Monday, 16:30 - 17:30 B-6-STU

Cloning of *Bacillus thuringiensis* plasmids using a modified plasmid capture system.

Qin Liu - Department of Agricultural Biotechnology, Seoul National University, Seoul, Seoul, Republic of Korea; ***Jong Yul Roh*** - Department of Agricultural Biotechnology, Seoul National University, Seoul, Seoul, Republic of Korea; ***Yong Wang*** - Department of Agricultural Biotechnology, Seoul National University, Seoul, Seoul, Republic of Korea; ***Jae Young Choi*** - Research Institute for Agriculture and Life

Sciences, Seoul National University, Seoul, Seoul, Republic of Korea; ***Hee Jin Shim*** - Department of Agricultural Biotechnology, Seoul National University, Seoul, Seoul, Republic of Korea; ***Hong Guang Xu*** - Department of Agricultural Biotechnology, Seoul National University; ***Xueying Tao*** - Department of Agricultural Biotechnology, Seoul National University,
Address for correspondence: liuyaqin0316@hotmail.com

Bacillus thuringiensis 1-3 (Bt 1-3), belonging to subsp. *aizawai* (H7), showed different characteristics in plasmid profiles and had *cry2A* gene in addition to *cry1Aa*, *cry1Ab*, *cry1C* and *cry1D*. This strain exhibited dual insecticidal activity against *Aedes aegypti* as well as *Plutella xylostella*. Recently, we improved the donor-s of plasmid capture system (PCS) by inserting *attB* sites including *lacZ* between transposable elements (designated as pPCS-Troy), to construct *E. coli-Bt* shuttle vectors. Through *in vitro* transposition with total plasmids DNA of Bt 1-3, 53 clones were acquired and their range of sizes was approximately 10 kb. Based on sequence analysis, they were classified in 4 groups showing similarity with 4 known plasmids, pGI1, pGI2, pGI3 and pBMB175, respectively. One of pGI3-like clones was fully sequenced and its open reading frames were analyzed. As a donor for construction of the shuttle vector, pDonrattPEm vectors harboring an erythromycin resistant gene between *attP* sites was constructed. Through BP recombination with pPCS-Troy-cloned Bt plasmids and pDonr-attPEm, an erythromycin resistant gene was transposed into Bt plasmids. This scheme proposes that *in vitro* transpositions using pPCS-Troy and BP recombination in pDonr-attPEm can be used to easily construct novel shuttle vectors with any Bt plasmids, and this combined procedure can introduce foreign genes into various circular DNA molecules.

Poster/Bacteria - Monday, 16:30 - 17:30 B-7

Effects of a new member of the toxin complex family on insects.

Sean Marshall - AgResearch, Lincoln, New Zealand; ***Sandra Jones*** - AgResearch, Lincoln, New Zealand; ***Mark Hurst*** - AgResearch, Lincoln, New Zealand
Address for correspondence: sean.marshall@agresearch.co.nz

The newly identified Enterobacteriaceae, *Yersina entomophaga*, was isolated from the New Zealand grass grub (*Costelytra zealandica*) in New Zealand soils. Host range testing of *Y. entomophaga* reveals it affects a broad range of insects, which includes pest species from Coleoptera, Lepidoptera and Orthoptera. Previous research has also demonstrated that *Y. entomophaga* secretes a protein complex termed the Super Toxin complex (STc). The STc complex is a member of the the growing Toxin complex (Tc) family of protein toxins, with other members being identified from several other bacterial species including *Serratia entomophila*, *Photorhabdus luminescens*, and *Xenorhabdus nematophilus*. The STc toxin has been purified and found to be highly effective against a range of insect species including grass grub and diamond back moth. Interestingly, STc treated grass grubs display an amber like gut colouration within 24-48 hrs. This is reminiscent of amber disease caused by Serratia entomophila strains bearing the *Sep* genes (Tc family member). However a major point of difference is that death occurs much more swiftly in STc exposed grass grubs (3-5 days) compared with amber diesese (a month or more). In an effort to better understand the host response to STc exposure, a histological study is being conducted on representative insect species that are susceptible to STc (i.e. grass grub and diamond back moth). We will discuss our results in relation to what is known from other members of the Tc family.

Poster/Bacteria - Monday, 16:30 - 17:30 B-8

Discovery of novel pesticidal protein genes in *Bacillus thuringiensis* using *de novo* sequencing.

Kimberly Sampson - Athenix Corp., Research Triangle Park, NC, USA; ***Ethan Dunn*** - Athenix Corp.; ***Jessica Zeigler*** - Athenix Corp.; ***Daniel Tomso*** - Athenix Corp.
Address for correspondence: ksampson@athenixcorp.com

Pesticidal proteins from *Bacillus thuringiensis (Bt)* have been identified primarily through empirical screening strategies, wherein bacterial strains that show bio-activity towards target pests are characterized to identify active proteins. Expression of bacterial genes, however, is influenced by numerous environmental stimuli, including culture media, O_2 concentration, temperature, and the presence of metabolites. Consequently, laboratory growth conditions are unlikely to induce the expression of all bacterial pesticidal genes, which can prevent detection of protein activity during screening. *Bt* strains also frequently contain multiple pesticidal genes, some of which can have overlapping spectrums of activity. Active proteins from strains having multiple pesticidal proteins may be difficult to isolate using activity-based screening and fractionation strategies. By applying a combination of *de novo* sequencing and bioinformatics analysis, we identified and cloned over 200 novel putative pesticidal proteins from *Bt* strains collected from a variety of environmental samples. These novel genes can be broadly categorized into several protein homology families: 3-domain delta endotoxins (103 genes), Mtx-like proteins (27 genes), putative binary proteins (16 genes), Vip-like proteins (11 genes), and others (60+ genes). As part of a discovery pipeline designed to identify genes with potential application in agriculture, many of these putative pesticidal proteins have been evaluated against several important agricultural pests. To date, we have identified new members of nearly every major group of the delta endotoxin family. Several novel proteins appear to represent new classes of proteins, and some appear to be fusions between known classes of pesticidal proteins. We are using these data to guide our discovery efforts, and to develop a refined model of pesticidal protein distribution across *Bt* strains.

Poster/Bacteria - Monday, 16:30 - 17:30 B-9

Screening and cloning of a *vip3A* gene from a *Bacillus thuringiensis* strain toxic to lepidopteran pests.

Joseilde O. Silva -Werneck - Embrapa Genetic Resources and Biotechnology, Brasilia, DF, Brazil; ***Elias F. Sabiá Júnior*** - University of Brasilia, Brasilia, DF, Brazil; ***Laíse S. Evangelista*** - University of Brasilia, Brasilia, DF, Brazil; ***Thatianny A.L. Silva*** - University of Brasilia, Brasilia, DF, Brazil; ***Vitor R. Valdez*** - University of Brasilia, Brasilia, DF, Brazil; ***Bergmann M. Ribeiro*** - University of Brasilia; ***Rose G. Monnerat*** - Embrapa Genetic Resources and Biotechnology,
Address for correspondence: joseilde@cenargen.embrapa.br

Some strains of *Bacillus thuringiensis* (Bt) produce Vip proteins during their vegetative growth, which are toxic to lepidopteran insects. Bt strains belonging to Embrapa Genetic Resources and Biotechnology *Bacillus* Collection, which have shown activity against lepidopteran insects, were screened by PCR for the presence of *vip3* genes. *vip3-like* genes were detected in eight out of 31 Bt strains analysed. Sequence analysis of the amplified PCR products confirmed their homology with *vip3A* genes. Proteins from supernatant of seleted Bt strains were extracted, analyzed by SDS-PAGE and bioassayed against three lepidopteran species: *Spodoptera frugiperda* (fall armyworm), *Anticarsia gemmatalis* (velvet caterpillar) and

Plutella xylostella (diamondback moth). SDS-PAGE analyses showed the presence of proteins around 85 kDa which is close to the expected size for *Vip3A* proteins. The extracted proteins showed very low levels of activity against *A. gemmatalis* and *P. xylostella* 2nd instar larvae. The proteins from two Bt strains showed activity against *S. frugiperda* 2nd instar larvae. A *vip3A* gene from the strain S711 was cloned and its protein will be analyzed and tested in bioassays against lepidopteran insects.

Poster/Bacteria - Monday, 16:30 - 17:30 B-10-STU

The Aealp is an important receptor molecule against Cry1AA.

Takaya Tomokiyo - Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan; ***Hisanori Bando*** - Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan; ***Shin-ichiro Asano*** - Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan
Address for correspondence: t-470er@abs.agr.hokudai.ac.jp

Bacillus thuringiensis subsp. israelensis (Bti) is toxic to different mosquito and black fly larvae. Bti produces six crystal toxins: Cry4Aa, Cry4Ba, Cry10Aa, Cry11Aa, Cyt1Aa and Cyt2Ba. The Cry11Aa has specific toxicity to *Aedes aegypti*, *Culex pipiens* and *Anopheles stephensi*. A key factor in determining the specificity of Bti toxins is receptor binding. Alkaline Phosphatase (ALP) is thought as a candidate receptor of Cry11Aa toxin in *Aedes aegypti* (Ae) midgut cells. An *alp* gene was cloned from midguts of *Aedes aegypti* larvae. The AeALP was subcloned into the expression vector pGEX and transformed into Escherichia coli to produce as a fusion protein (GST-AeALP). The expressed GST-AeALP showed ALP activity. GST-AeALP blotted on to PVDF western blotting membrane was probed with biotinylated Cry11Aa. To confirm the importance of this interaction, A. aegypti larvae were fed with Cry11Aa toxin alone or in combination with GST-AeALP. In consequence, Cry11Aa toxicity attenuates in the presence of GST-AeALP. Expression of a recombinat AeALP in *Spodoptera frugiperda* (Sf9) cells made them sensitive to Cry11Aa, providing evidence that the AeALP plays a role in Cry11Aa toxicity. Our studies reveal that the AeALP is an important receptor molecule against Cry11Aa.

Poster/Bacteria - Monday, 16:30 - 17:30 B-11

Virulence causing ability of *Serratia proteamaculans* strains towards the grass grub *Costeyltra zealandica.*

Mark Hurst - Agresearch, Lincoln, Canterbury, New Zealand; ***Maureen O'callaghan*** - Agresaerch, Lincoln, Canterbury, New Zealand; ***Joanne Calder*** - Agresearch, Lincoln, Canterbury, New Zealand; ***Lincoln Harper*** - Agresearch, Lincoln, Canterbury, New Zealand
Address for correspondence: mark.hurst@agresearch.co.nz

Some strains of *Serratia entomophila* and *S. proteamaculans* cause amber disease of the New Zealand grass grub *Costelytra zealandica* (Coleoptera: Scarabaeidae). The disease determinants are encoded on a large 153,404-bp plasmid, termed pADAP for amber disease associated plasmid. Occasionally field isolated *C. zealandica* larvae of an unusual colouration are observed. Bacteria were isolated from these larvae and assessed for ability to induce disease in grass grub. Eight strains causing unusual disease phenology were isolated. 16sRNA DNA sequence analysis identified all strains as S. proteamaculans. In bioassays, 8 strains caused high mortality of grass grub within a 7 day period. Although these strains caused grubs to die more quickly than *S. entomophila*, only 60-90 % of larvae died, while the remaining larvae did not become diseased. Two further strains were identified that exhibited 100% mortality within a seven day duration. All 10 strains contained a plasmid which were shown to be variants of pADAP. The DNA sequence of plasmid-encoded virulence determinants of one of the S. proteamaculans strains (143), that exhibits a pathotype of only 60-70% of *C. zealandica* larvae succumbing to disease was determined. The *S. proteamaculans* strain 143 virulence determinants shared high DNA similarity to the pADAP sep virulenceassociated region, with DNA sequence variation in the sepA gene and the variable region of the sepC component. No similarity to the pADAP antifeeding prophage orthologue was identified. Through comparison of the DNA sequence to that of pADAP, we define the possible origins of the S. entomophila pADAP virulence determinants.

Poster/Bacteria - Monday, 16:30 - 17:30 B-12-STU

Insecticidal activity and mode of action of Cry8d against Japanese beetle.

Takuya Yamaguchi - Hokkaido Univ., Sapporo, Hokkaido, Japan; ***Ken Sahara*** - Hokkaido Univ., Sapporo, Hokkaido, Japan; ***Hisanori Bando*** - Hokkaido Univ., Sapporo, Hokkaido, Japan; ***Shin-ichiro Asano*** - Hokkaido Univ., Sapporo, Hokkaido, Japan
Address for correspondence: yamagu@abs.agr.hokudai.ac.jp

Bacillus thuringiensis (Bt) is a rod shaped, Gram positive, spore-forming bacterium. Bt produces parasporal crystal proteins during sporulation. Since the crystal proteins often show insecticidal activity to various harmful pests of Lepidoptera, Diptera and Coleoptera orders, Bt is widely used in pest control agents. Larvae of the Japanese beetles live in soil and cause great damages to the roots of turf and other vegetations. On the other hand, adults damage the leaves, flowers and fruits but not roots. If there is a Bt-based insecticide that can control both larvae and adults of the beetles, more effective insect control is possible with this agent than the others that are toxic to only the larvae. We screened from our Bt culture collection, then identified that SDS-502 and BBT2-5 show insecticidal activity against both larvae and adult of Japanese beetle. Two Cry genes, *Cry8Da* of SDS-502 and *Cry8Db* of BBT2-5 were cloned and expressed in an acrystalliferous *B. thuringiensis* strain BT51. The insecticidal activities of *Cry8Da* and *Cry8Db* against larvae and adults of Japanese beetle were tested. Both *Cry8Da* and *Cry8Db* had toxicities against larvae and adults. The activation processes of *Cry8Da* and *Cry8Db* by adult and larval gut juice were analyzed. *Cry8Da* and *Cry8Db* produced a toxic core of approximately 70 kDa and 58 kDa equally. Further, a co-precipitation assay was used to investigate the binding property of the trypsine activated *Cry8Da* and *Cry8Db* to Japanese beetle's BBMVs. Is indicated that *Cry8Da* and *Cry8Db* bound to receptor molecules specifically.

Poster/Bacteria - Monday, 16:30 - 17:30 B-13

Expression of a Cry1ac-gfp fusion protein in *Bacillus thuringiensis.*

Hui Yang - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Fuping Song*** - Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Jie Zhang*** - Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Dafang Huang*** - Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China
Address for correspondence: yanghui5020801@163.com

For the study of cry gene expression in the *Bacillus thuringiensis*

crystalnegative strain HD-73< sup>-, the *cry1Ac* gene controlled by the Bt I and Bt II promoters was fused with *gfp* gene at the 3'-terminal end and cloned into the pHT315 vector resulting in the pHT-CG1 plasmid. Western blot analyses indicated that strain HD73- carrying pHT-CG1 produced the 160 kDa Cry1Ac-GFP fusion proteins. Using laser confocal microscopy we observed that the Cry1Ac-GFP fusion proteins were dispersed throughout the cell prior to and during forespore septum formation. Bioassay results demonstrated that the Cry1Ac-GFP fusion proteins were toxic to *Plutella xylostella* larvae. In addition, our results showed that expression of *cry1Ac* gene occurred much earlier than septum formation. It indicated that *cry1Ac* gene was expressed at the late transition phase. It suggested that unknown factors which were expressed earlier than ?< sup>E might be involved in the regulation of *cry* gene expression. These results may reflect the complicated *cry* expression and regulation systems.

Poster/Bacteria - Monday, 16:30 - 17:30 B-14

Research on *Bacillus thuringiensis* against animal and plant parasitic nemoatodes.

Ziniu Yu - State Key Laboratory of Agricultural Microbiology, National Enginerring Research Center for Microbial Pesticides, Wuhan, Hubei Province, People's Replublic of China; ***Jianhong Li*** - State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Wuhan, Hubei Province, People's Replublic of China; ***Mingshun Li*** - State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Wuhan, Hubei Province, People's Replublic of China; ***Ming Sun*** - State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Wuhan, Hubei Province, People's Replublic of China
Address for correspondence: jianhl@mail.hzau.edu.cn

Animal and plant parasitic nematodes and entomopathogenic nematodes belong to the *Nemathelminthes*. However, their effects are different. The parasitic nematodes cause tremendous losses to the animal and crops, while the entomopathogenic nematodes use as a biological control agent for controlling pest, and increasing the crop yield. Currently, *Bacillus thuringiensis* agent is using as the world's most successful microbial pesticide. In recent years, the study found that *B.thuringiensis* shows cytotoxicity to animal and plant parasitic nematodes and it leads a new way to prevent and treat animal and plant parasitic nematodes. The harm of animal and plant parasitic nematodes. Plant-parasitic nematode is one of the main diseases on crops and forests. It lives in various parts of plant and stops the growth of plant resulting in destruction of plant organizations. Animal nematodes seriously affect the growth of livestock. All of them cause huge losses to the production of agriculture, forestry, and livestock. Until now, there are 10 genus of the most damaging parasitic nematodes of animals and plants found in worldwide. There are many kinds of controlling methods have been applied on prevention of animal and plant parasitic nematodes. Physical control methods having significant limitations in the prevention and treatment of them. Chemical nematicides cause impacts to natural environment and threat the health of human beings. Biological control methods against them mainly use parasitic fungi or bacteria, and the prevention and treatment of biological control methods are affected by regional and specificity of host. The active ingredient of *Bacillus thuringiensis.* According to the differences of serological response to the flagellar antigens, *B. thuringiensis* can be divided into 72 serotypes and 84 subspecies. Its main active ingredients against pest have ICPs, thuringiensin, Zwa, Vip, enzymes and so on. The *cry* genes encoding ICPs are divided into 55 groups and more than 400 sub-categories. The thuringiensin (e.g ?-exotoxin) is a nucleoside antibiotic with broad-spectrum insecticidal activity. The research of *Bacillus thuringiensis* against animal and plant parasitic nematode. In early 1970s, the toxicity of thuringiensin to the eggs and larvae of *Meloidogynidae spp.* has been found. Further studies found that the toxicity of parasporal crystals against the nematodes as well. According to similarity of their amino acid sequence, the *cry* genes encoding ICPs can be divided into two classes. The mechanism of ICPs effecting on nematodes is to separate its gut from its body wall, eventually result in death of nematode. However, its effects on the eggs of nematodes have not been documented. Therefore, further study on *B. thuringiensis* against parasitic nematodes of animals and plants is needed.

Poster/Bacteria - Monday, 16:30 - 17:30 B-15

Characteristics of a *B. thuringiensis spoIIID* gene mutant.

Qianqian Zhang - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Junlan Ma*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Fuping Song*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Jie Zhang*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Dafang Huang*** - Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China
Address for correspondence: jiezhang@caas.net.cn

SpoIIID is a small, sequence-specific DNA-binding protein that activates or represses transcriptions of many genes in sporulation. During sporulation of *Bacillus subtilis*, four regulatory proteins act in the order -E, *SpoIIID*, -K, and GerE to temporally control gene expression in the mother cell. Previous study showed that *cry1*-type genes were transcribed by two overlapping promoters (BtI and BtII) controlled by RNA polymerase containing sporulation sigma factors -E and -K in *B. thuringiensis*. This study focused on the effect of formation of spores and expression of the cry gene by deletion of a *spoIIID* gene in *B. thuringiensis*. The *spoIIID* gene inactivation mutant was obtained by homologous recombination and its genetically complementary strain was also constructed. Scanning electron microscopy and spore formation analysis showed that nearly no spores were produced in the strain with *spoIIID* deletion while the crystal existed. SDS-PAGE results showed that the expression of a cry gene was decreased in the mutant in LB medium and not affected in SM medium. Beta-galatosidase assays indicated that transcriptional activity of pro-K was decreased and that of pro-E was increased in the mutant strain by fusions between two promoters and lacZ gene.

Poster/Bacteria - Monday, 16:30 - 17:30 B-16

Transcriptomic and proteomic analysis of *Bt*-resistant *Spodoptera frugiperda* sf9 cell lines.

Claude Castella - INRA, Sophia Antipolis, France; ***Pierre Barbero*** - INRA, Sophia Antipolis, France; ***Trang Tran*** - INRA, Sophia Antipolis, France
Address for correspondence: pauron@sophia.inra.fr

Sf9 originally derives from ovarian tissue of the Lepidoptera *Spodoptera frugiperda* and is one of the few insect cell lines available for heterologous expression studies using baculovirus or stable transfection. It has also been previously demonstrated that Sf9 cells are susceptible to the toxic effect

of the delta endotoxin Cry1C produced by *Bacillus thuringiensis* (Kwa et al., 1998). We have measured the toxicity parameters of Cry1C on Sf9 cells which were then cultured in the presence of toxin at the LC50 or LC80. After several passages, cells were found able to resist to the lethal effect of the toxin. Hence we recovered RNAs from LC50 and LC80 cells as well as from untreated Sf9 cells which were grown in parallel. We have compared the gene transcription patterns of the various cell populations using a cDNA array which has been specifically developed for Spodoptera. We have also performed 2-D electrophoresis of the membrane and cytosolic proteomes obtained in each experimental condition. We will report preliminary data obtained by both analysis techniques and present several putative candidates which might be involved in the molecular mode of resistance of Sf9 cells to Cry1C. Référence Kwa MS, de Maagd RA, Stiekema WJ, Vlak JM, Bosch D (1998). Toxicity and binding properties of the *Bacillus thuringiensis* deltaendotoxin Cry1C to cultured insect cells. J Invertebr Pathol, 71:121-127.

Poster/Bacteria - Monday, 16:30 - 17:30 B-17

Cross-resistance of Cry1ac-selected Asian corn borer to other Bt toxins.

Hailiang Han - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China; ***Kanglai He*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China; ***Guangtao Li*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China; ***Zhenying Wang*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China; ***Jie Zhang*** - State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, P. R. China
Address for correspondence: kanglai.he@263.net

Asian Corn Borer (ACB) *Ostrinia furnacalis* (Guenée) is the most important pest targeted by transgenic Bt (*Bacillus thuringiensis*) maize in China. However, the evolution of insect resistance will threatened the long term use of Bt maize. The exotic and domestic Bt maize incorporated with several Bt genes into maize genomes have been assessed for environment safety although tests for cross-resistance among different toxins have been limited by a lack of resistant colonies. A resistant strain of Asian corn borer, ACB-AcR, selected with Cry1Ac protein incorporated into artificial diet developed significant levels of resistance (14-fold) after 27 generations. Additionally, ACB-AcR maintained on a constant concentration (8?g/g) was developed 48.9-fold resistance at generation 82. LC50s of Cry1Ab, Cry1Ah, and Cry1Ie were measured to ACB-AcR and ACB-BtS stains. Significantly, the greatest cross-resistance was observed to Cry1Ah with resistance ratios up to 14.9-fold. Low levels (6.3-fold) of cross-resistance were detected with Cry1Ab. In contrast, Cry1Ie susceptibility was unaffected by selection with Cry1Ac. These results indicate that the availability of multiple toxins could improve resistance management strategies, provided cross-resistance among toxins is not a factor.

Poster/Bacteria - Monday, 16:30 - 17:30 B-18

Characterization and organization of novel *cry* genes isolated from mosquitocidal *Bacillus thuringiensis* strain.

Yu Karatani - Kinki University, Kinokawa, Wakayama, Japan; ***Akiyoshi Kasugai*** - Kinki University, Kinokawa, Wakayama, Japan; ***So Takebe*** - Kinki University, Kinokawa, Wakayama, Japan
Address for correspondence: takebe@waka.kindai.ac.jp

We isolated a novel *Bt* strain, TK-E6, from grove soil in Japan. The crystal of this strain showed strong mosquitocidal activities to *Aedes aegypti* and *Anopheles quadrimaculatus* larvae, but weak to *Culex pipiens* larva. A degenerate PCR based gene-screening method, that we developed, detected more than ten *cry* genes in this strain. Nucleotide sequences of these genes were determined and DNA-deduced ORFs encoding 140 – 145 kDa Cry proteins were cloned into a *Bt* expression vector carrying *cyt1A* promoter and *cry4A* terminator. The recombinant plasmids were introduced into a crystal minus mutant *Bt* cell (BGSC No. 4Q7 strain). Each Cry protein was purified by NaBr gradient centrifugation method, and used for mosquitocidal assay against *Ae aegypti* larva, any protein, however, did not show the strong mosquitocidal activity when used alone. These results suggested that there was a synergistic action with some proteins. *Bt.* TK-E6 has at least five large plasmids, their nucleotide sizes are over 100 kbp. Elucidation of structure of the *Bt.* TK-E6 large plasmids is very important to know evolution of *Bt* and *cry* genes. Therefore, in this study, we analyzed gene organization of large 5 plasmids from *Bt.* TK-E6. Southern hybridization experiments revealed that ten genes had been distributed to five large plasmids. Interestingly, each gene did not exist in two plasmids or more. Moreover, the chaperone-like protein, and ISs (insertion sequences) and transposon structure are also found in the upand downstream of all genes. It is very possible that some DNA rearrangement of gene amplification occurred in both intra- and interplasmids during the evolutional process of *Bt*. TK-E6.

Poster/Bacteria - Monday, 16:30 - 17:30 B-19

Candidatus liberibacter asiaticus **propagation in psyllid cell cultures.**

Mizuri Marutani-Hert - USDA-ARS, Fort Pierce, Florida, USA; ***Wayne Hunter*** - USDA-ARS, Fort Pierce, Florida, USA; ***David Hall*** - USDA-ARS, Fort Pierce, Florida, USA
Address for correspondence: Wayne.hunter@ars.usda.gov

The first psyllid cell culture was developed and screened as a living medium for the culturing of *Can. Liberibacter asiaticus*, CLas. Since CLas is considered to be a psyllid pathogen that replicates within the psyllid, but has so far remained unculturable in vitro, we chose to set up a new approach using psyllid cell cultures as the media to isolate and culture this bacterium. The Asian Citrus Psyllid, *Diaphorina citri*, (Hemiptera: Psyllidae) is a highly competent vector of the phloem-inhabiting bacterium CLas, associated with the disease Huanglongbing, HLB. The HLB disease causes reduction of fruit yields, fruit quality, and ultimately tree death. World-wide HLB has become the major limiting factor to the production of citrus. Commercially available insect cell culture solutions/media were used to produce a psyllid cell culture medium. The media were screened for viability to culture cells/tissues from AsCP embryos and midgut tissues. Multiple cells cultures were established and two media were defined HH-50, and HH-70. Cell lines have been maintained for over 6 months. Real-time PCR provided evidence that CLas has remained viable and replicated within the psyllid cell cultures. Success may depend on the co-cultivation with a secondary bacterium. Creation of psyllid cell cultures provides another system to examine CLas replication, and psyllid viral pathogens, in a highly defined system.

Poster/Bacteria - Monday, 16:30 - 17:30 B-20

Metagenomic approach to psyllid microbes.

Mizuri Marutani-Hert - USDA-ARS, Fort Pierce, Florida, USA; ***Wayne Hunter*** - USDA-ARS, Fort Pierce, Florida, USA; ***David Hall*** - USDA-ARS, Fort Pierce, Florida, USA
Address for correspondence: Wayne.hunter@ars.usda.gov

A metagenomics approach was used to identify the bacterial community in the Asian citrus psyllid, *Diaphorina citri* (Hemiptera). Huanglongbing (HLB) is transmitted by *D. citri*, and is the most severe diseases of citrus crops. One strategy to manage HLB is aimed at suppression of psyllid populations. Insects such as psyllids within the Hemiptera, feed from the phloem of plants ingests a diet which is rich in carbohydrates but deficient in essential amino acids. These insects support maternally inherited bacterial mutualists, referred to as endosymbionts. The endosymbionts live within bacteriocytes and are generally thought to supplement the psyllid's nutrition. To investigate the endosymbiotic microbiota of *D. citri*, we analyzed eubacterial 16S-23S rDNA amplified from psyllids and identified transcripts from a cDNA psyllid library made from field collected adults feeding from citrus. These data suggest psyllids are supported by 3-4 endosymbiotic bacteria, a *Wolbachia*, and at least 4 other bacteria, which demonstrates a rich bacterial fauna which may have important interactions between each other and most likely with *Liberibacter asiaticus* when it occurs in psyllids.

Poster/Bacteria - Monday, 16:30 - 17:30 B-21-STU

Characterisitics of a *Bacillus thuringiensis* strain fcd114.

Xiaodong Sun - State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Changlong Shu*** - State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***JiGuo Gao*** - Northeast Agricultural University, HarBin, Heilongjiang, China; ***Fuping Song*** - State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China; ***Jie Zhang*** - State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China
Address for correspondence: jzhang@ippcaas.cn

A new *Bacillus thuringiensis* strain, FCD114, was isolated from HeBei soil samples in China. It produced spherical parasporal inclusions similar to that of the *B. thuringiensis* subsp. *japonensis* Buibui strain which showed toxicity to both *Anomala corpulenta* and *Popillia japonica.* The plasmid profile revealed that Bt FCD114 contained six bands. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis showed one major band with an estimated molecular mass of 130 kDa. Polymerase chain reaction–restriction fragment length polymorphism results showed that a novel cry8-type gene sequence was found in the FCD114 strain. We cloned and sequenced this novel gene. The nucleotide sequence data of this gene were assigned GenBank accession numbers FJ198072. Sequence analysis showed that this gene protein consisted of 1157 residues of amino acids with a predicted molecular weight of 130kDa and isoelectric point pH 4.86. Bioassay results showed that FCD114 had no toxicity against several Coleopteran and Lepidopteran pests. However, FCD114 exhibited toxicity against larvae of *Leptinotarsa decemlineate* and *Holotrichia oblita.* This is the first report of the occurrence of a *Bacillus* strain that has insecticidal activity against larvaes of both *Holotrichia oblita* and *Leptinotarsa decemlineate.*

Poster/Bacteria - Monday, 16:30 - 17:30 B-22

withdrawn

Poster/Bacteria . Monday, 16:30 - 17:30 B-23

Toxicity of *Bacillus thuringiensis* and its combined effect with *Habrobracon hebetor* to *Plodia interpunctella.*

Akinkurolere Rotimi Oluwafemi - Huazhong Agricultural University, Wuhan, China, Wuhan, Hubei, People's Republic of China; ***Hong-Yu Zhang*** - Huazhong Agricultural University, Wuhan, China, Wuhan, Hubei, People's Republic of China
Address for correspondence:

Through bioassays, isolate IMM-408 was found to have highest toxicity to *P. interpunctella* among 122 isolates from warehouse and *B. thuringiensis* serovar *kurstaki* HD-1, and was selected and analyzed in detail in this study. Isolate IMM-408 contained cry1Ab9, cry1Ca1, cry1Da1 and cry2 gene, produces ~135 kDa crystal protein and belonged to serotype H7. The LC50 of isolate IMM-408 for P. interpunctella was 1.24 μg/ml. Furthermore, the suitability of combining microbial pesticides and natural enemies for integrated pest management of stored cereal was evaluated. *B. thuringiensis* or *H. hebetor* alone caused significantly lower *P. interpunctella* larval mortality (41.67% and 35.35% respectively) than the combination treatment (86%). However, more eggs were significantly laid by *H. hebetor* on hosts placed in control than on hosts in *B. thuringiensis* contaminated diets. Hatchability of *H. hebetor* eggs were not affected in all treatments. Fewer wasps emerged from *B. thuringiensis*-parasitoid combine treatment than in none *B. thuringiensis* treatments. Although the size of adult parasitoids that emerged from combine treatments appeared smaller than those in the control, their developmental period was not significantly different. Similarly, no significant difference was observed in the longevity of adult wasps emerging from both treatments. The sex ratio of *H. hebetor* progeny and this trend is similar to the observations in combine treatment Therefore, since *B. thuringiensis* did not prevent parasitoid development, suggesting that a combine treatment with *B. thuringiensis* and parasitoid release, would produce better protection against *P. interpunctella*. Acknowledgements: This research was supported by China National Science and Technology Project of the 11th Five-Year Plan (No. 2006BAI09B04-06 and No. 2006BAD02A18-03), and Hubei Key Project of International Cooperation on Science and Technology (No. 2006CA005).

MICROBIAL CONTROL

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-1

A new microbial insecticide from *Chromobacterium subtsugae.*

Lisa Chanbusarakum - Marrone Organic Innovations, Davis, CA, USA; ***Eunice Tan*** - Marrone Organic Innovations, Davis, CA, USA; ***Ratnakar Asolkar*** - Marrone Organic Innovations, Davis, CA, USA; ***Huazang Huang*** - Marrone Organic Innovations, Davis, CA, USA; ***Pam Marrone*** - Marrone Organic Innovations, Davis, CA, USA
Address for correspondence: lchan@marroneorganics.com

Marrone Organic Innovations has licensed a technology based on a novel species of *Chromobacterium* from USDA and is developing it into a microbial bioinsecticide. The development work includes media optimization to maximize the yield of secondary metabolites responsible for insecticidal activity as well as formulation development for increased efficacy and storage stability. Bioactive compounds are extracted from fermentation broths and the resulting crude extracts are fractionated for compound isolation and identification. Our studies confirm the previous data from USDA; the insecticidal activity of fermentation broths develops over time and coincides with the cell death during the stationary growth phase. Cell-free extracts have good activity against insect pests.

The active compounds in the whole-cell broth seem to be heat-stable but some activity is lost during freeze drying. Work is in progress for media optimization, formulation development and active compound identification. Spectrum testing against various insect pests is continuing through bioassays as well as greenhouse and field studies.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-2

Lethal encounters in *Synanthedon* tunnels: apple clearwing moth larvae and entompthogenic fungi and nematodes.

Joan Cossentine - AAFC, Summerland, BC, Canada; ***Paul Randall*** - AAFC, Summerland, BC, Canada; ***John Bissett*** - AAFC, Ottawa, ON, Canada; ***Lerry Lacey*** - USDA-ARS, Wapato, WA, USA
Address for correspondence: Joan.Cossentine@agr.gc.ca

The apple clearwing moth (ACWM), *Synanthedon myopaeformis*, became established in commercial apple orchards in southern British Columbia in 2005. This invasive European species presents a control challenge to the tree fruit industry in the Pacific Northwest as heavy infestations have the capability of causing significant debility or death to young trees. The moths emerge over a three month period to lay eggs on damaged bark near graft unions and pruning cuts and the larvae bore into the tree, creating tunnels in the cambium. The cryptic larvae develop over two years which makes timely treatment difficult. Orchard collected larvae were found to host an indigenous entomopathogenic fungus which has been identified as *Metarhizium brunneum* (Petch). Laboratory bioassays found late instar ACWM larvae to be similarly susceptible to infection and mortality by both the *M. brunneum* and the GHA isolate of *Beauveria bassiana*. Overwintered ACWM larvae were exposed to both entomopathogenic fungi and nematodes in field trials using trunk treatments. The potential for ACWM larvae to succumb to an EPF infection and/or entomopathogenic nematodes in the larval tunnels is discussed.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-3

Formulation and evaluation of aquatic mosquito larvicides based on *Bacillus thuringiensis subsp. israelensis* in Vietnam.

Binh Dinh Ngo - Institute of Biotechnology, Hanoi, Hanoi, Vietnam; ***Tuan Dinh Nguyen*** - Institute of Biotechnology, Hanoi, Hanoi, Vietnam; ***Ha Thu Trinh*** - Institute of Biotechnology, Hanoi, Hanoi, Vietnam; ***Thanh Quang Le*** - Ministry of Science and Technology, Hanoi, Hanoi, Vietnam
Address for correspondence: binh.gen@gmail.com

From more than 1000 *Bacillus thuringiensis* strains in the Vietnam Bt Collection, a number of 75 strains were screened and identified to be classified into two H serogroups *israelensis* and *morrisoni.* These strains then were screened by bioassays against three important disease transmission vectors: *Anopheles minimus, Aedes aegypti, Culex quinquefasciatus.* Results of screening showed that there were 28, 29, 30 strains have high toxicity, from 81 – 100 % mortality to *An. minimus, Ae. aegypti, C. quinquefasciatus,* respectively. The *B. thuringiensis* serovar *israelensis* Bti-11, high toxicity to the three examined mosquito larvae, was chosen for production of mosquito larvicides. The formulations were made in the small round cake form with 3 cm diameter, 2-5 mm thickness. The used raw materials are cheap and available in Vietnam including corn cob, sugar cane bagasse, cork, popcorn and some new nanotech materials. Results showed that the formulation made of corn cob (F1), the formulation made of sugar cane bagasse and polyvinyl alcohol adhesive (F4) got the highest efficacy, 96.6 and 100 % respectively. The F4 formulation was remarkably degraded, components and substrates took apart after 11 days, while the formulation F1 was not noticeably corrupted. For long-term residual activity of products, experiments were designed with natural conditions reproduced, the F1 and F7 formulations were revealed to have high efficacy maintenance, larva mortality of F1 was 97.03 % after 63 days and data were recorded continuously until day 100th. The formulation F1 was used for field trial in some water still ponds in urbanizing areas where natural mosquito density is usually very high in some districts of Hanoi city. Mosquito larva density in experimental ponds reduced with ratio from 90.0 to 100 % after 72 hours of treatment. This mosquitocidal product, corn-cob dunks, was accepted by the National Office of Intellectual Property of Vietnam.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-4

Shelf-life of *Beauveria bassiana* conidia in gaseous atmospheres under high temperature regimes.

Marcos Faria - Cornell University, Ithaca, NY, USA; ***Ann Hajek*** - Cornell University, Ithaca, NY, USA; ***Joseph Hotchkiss*** - Cornell University, Ithaca, NY, USA; ***Stephen Wraight*** - USDA-ARS, Ithaca, NY, USA
Address for correspondence: steve.wraight@ars.usda.gov

The retention of high viability during storage is essential for effectiveness and thus market acceptance of fungus-based biopesticides. The length of adequate shelf-lives for mycoinsecticides is controversial, with proposed requirements varying from a few weeks to 18 months. Shelf-life determinations under non-refrigerated conditions, especially high temperature regimes common in tropical regions (30–50 ºC), deserve growing attention. Most previous studies dealing with high ambient temperatures have involved storage environments where important parameters such as temperature and water activity (aw) were uncontrolled. To our knowledge, the longest reported survival (retention of > 80% viability) of *Beauveria bassiana* under tropical conditions was 80 days at 40 ºC and 17 days at 50 ºC (Hong et al., 2001; Mycol. Res. 105, 597-602). In this study, we investigated effects of modified storage atmospheres on longevity of *B. bassiana* strain GHA conidia. Decreasing O2 concentrations in storage increased survival. In experiments carried out at 50 ºC for 33 days, 0.100 aw conidia stored in 0.3, 2.5, 4.8, 12.3 and 22.4% O2 (balanced with N2) showed 69, 64, 55, 27 and 16% germination (within 24 h at 25 ºC), respectively. Replacement of O2 with CO2, N2, He, H2, or various mixtures of these gases provided similar increases in conidial longevity. At 40 ºC, viability of dry conidia was 89% after 3 months but dropped to 48% after 180 days in a CO2-rich atmosphere. At 50 ºC, viabilities for conidial powders with 0.100 aw were ca. 80% following 33 days at 50 ºC, but significantly less (43-54%) for powders with 0.140 aw. Significant air leakage into our storage containers (glass canning jars) was detected after a few months of storage, and we believe that results could be substantially improved through use of truly hermetic packages.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-5

Conserving and using entomopathogenic fungi and nematodes within Chile.

Andrés France - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Merino Loreto*** - INIA Quilamapu, Chillán, Chile; ***Edgington Steve*** - CABI, Egham, Egham, UK; ***Dave Moore*** - CABI, Egham, Egham, UK; ***Marcos Gerding*** - INIA Quilamapu, Chillán, Chile
Address for correspondence: afrance@inia.cl

The Insect Pathology Program of the National Institute of Agricultural Research (INIA) Quilamapu Research Station, in Chile, is working in collaboration with CABI (UK) on a Darwin Initiative (DEFRA, UK) to conserve and use Chilean entomopathogenic (EP) microorganisms. The aim of this collaboration was to collect EP fungi and nematodes from some of the major ecological habitats in Chile. Six survey transects were chosen: 1) Latitude 20º, from the sea to the Andes Altiplano and on the periphery of the Atacama desert; 2) Latitude 30º, Atacama desert with remnants of ancient tropical forests; 3) Latitude 33º, a Mediterranean vegetation area; 4) Latitude 37º, a transitional zone from dryland to wetland; 5) Latitude 46º, zone of heavy rainfall, relatively cold, with humid forests and pampas areas, 6) Latitude 52º, Tierra del Fuego, with near Antarctic weather and flora and fauna adapted to low temperatures. These transects have been surveyed, revealing 528 isolates of EP fungi, predominately *Metarhizium* and *Beauveria* spp., and 101 isolates of EP nematodes between *Heterorhabditis* and *Steinernema* spp. This collection is allocated in the Genetic Resource Collection of INIA, significantly enhancing the bank of indigenous germplasm already present. It is likely that indigenous isolates will show stronger adaptations to Chilean conditions, compared to exotic ones, and provide important pest control agents. Financement: Darwin Initiative, DEFRA UK

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-6

Aphid lethal paralysis virus, a potential biological control agent.

Liljana Georgievska - Iowa State University, Ames, IA, USA; ***Sijun Liu*** - Iowa State University, Ames, IA, USA; ***W. Allen Miller*** - Iowa State University, Ames, IA, USA; ***Bryony Bonning*** - Iowa State University, Ames, IA, USA
Address for correspondence: liljanag@iastate.edu

Aphids cause enormous economic damage to virtually all crops, through direct injury and as vectors of plant viruses. Current management is based primarily on the frequent application of environmentally damaging insecticides. Aphid populations are also renowned for rapid development of insecticide resistance. Aphid lethal paralysis virus (ALPV; Dicistroviridae) is a positive-strand RNA virus that paralyses the aphid host. ALPV is known to infect eight aphid species, including the green peach aphid, *Myzus persicae* (Sulzer) and several pests of small grains. Here we report that ALPV is also infectious to the pea aphid, *Acyrthosiphon pisum* (Harris). Purified ALPV fed to third instar *A. pisum* nymphs significantly reduced the lifespan of the aphids, which were paralyzed and fell from the host plant. The presence of negative strand RNA in virus-infected aphids, which is indicative of virus replication, was confirmed by RT-PCR. We also present a diagnostic qRT-PCR assay for virus detection and for quantification of virus load in different aphid tissues. On the basis of high pathogenicity and infectivity against a broad range of aphid species, ALPV shows promise for use as a biological control agent.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-7

Entomopathogenic fungus *Myriangium* sp.: biology and possibilities for mass-production.

Svetlana Gouli - University of Vermont, Burlington, Vermont, USA; ***Bruce Parker*** - University of Vermont, Burlington, Vermont, USA; ***Margaret Skinner*** - University of Vermont, Burlington, Vermont, USA; ***Vladimir Gouli*** - University of Vermont, Burlington, Vermont, USA
Address for correspondence: sgouli@uvm.edu

Fungi from the genus *Myriangium* are known as important regulating factors of scales in nature. Diagnostics of mycoses caused by these fungi and isolation of pathogens in the pure culture are sufficiently difficult problem. The difficulties are linked with two principal circumstances. In the first place, the fungi from the genus *Myriangium* are characterized very slow speed of growth, and infection process become often complicated with additional microbial contaminants characterizing high speed growth. In the second place, fungi do not form fruiting bodies for long time periods, and, as a rule, identifications can be made only based on DNA analysis. Determination of the principal properties of these fungi is essential for theirs practical application for the control of scales and other noxious arthropods. Isolation of fungus *Myriangium* sp. was done from scale, *Fiorinia externa,* and pure cultures were identified using DNA analyses (Marcelino et al., 2009. J. Appl. Entomology. 133). Optimal conditions for submerged cultivation of the fungus on liquid mediums were established. Potato-dextrose medium was the most productivity for submerged cultivation of fungus. Submerged cultivation of fungus was conducted for the period of 72, 144 and 288 hours. The fungal biomass was separated by centrifuge throughout 10 minutes at 1500 rev. /min at 2-40C, and sediment was dried. Production of fungal dry biomass was following: 2.8±03 g/l, 3.5±0.5 g/l and 4.1±0.7 g/l in accordance with period of cultivation. Augmentation of fungal biomass was observed after all cultivation periods. Millet grain and corn starch materials were evaluated for processing of biomass for obtaining fungal cryptogamic material. During the second stage of cultivation the fungus was completely covered the millet grains and formed numerous micro-sclerotia without any fruiting morphological structures. Growth and development of fungus on starch granules was less productive in compare with millet grains.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-8-STU

Identifying potential microbial control agents in Ghana (rationalising pesticide use against heteropteran pests).

Nick Jessop - Imperial College, Ascot, Berkshire, UK; ***Roy Bateman*** - Imperial College, Ascot, Berkshire, UK
Address for correspondence: nick.jessop@imperial.ac.uk

Insect pests, such as capsids (Hemiptera: Miridae), are amongst the most important constraints to cocoa production in Ghana, and West Africa in general. Although they can often be controlled with insecticides, application has to be frequent and sustained, which is not only expensive but, potentially, environmentally damaging. Moreover, intensified pesticide residue legislation in key markets such as the EU and Japan, is driving research into alternative control techniques. One promising possibility is the use of entomopathogenic fungi for their control, but to date sufficiently virulent isolates have yet to be found. A Royal Society sponsored project has enabled a network of Ghanaian and UK scientists to review methods of collecting and developing new isolates. The project addressed the national research priority areas of agricultural sustainability by reduction of crop losses, and exploitation of local biodiversity. Wild cola (*Cola cordifolia*) has been suggested as an original native host of cocoa mirids (Entwistle). An area of wild cola was sampled for Heteropteran species using a combination of canopy fogging and pheromone lures. Sampled insects were then screened for possible isolates.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-9

Influence of polyoxyethylene-(3)-isotridecyl ether as a spreader on the degradation of two species of aphid cuticles by *Beauveria bassiana* SFB-205 supernatant

Jae-Su Kim - Entomology Research Laboratory, University of Vermont & Dongbu HiTek, Burlington, VT, USA; ***Jae-Su Kim*** - Agricultural Research Institute, Dongbu HiTek Co., Ltd., Daejeon, South Korea;

Jong Yul Roh - School of Agricultural Biotechnology, Seoul National University, Seoul, South Korea; ***Yeon Ho Je*** - School of Agricultural Biotechnology, Seoul National University, Seoul, South Korea
Address for correspondence: jae-su.kim@uvm.edu

Applications of *Beauveria bassiana* SFB-205 (KCCM 10892P) supernatant (10 ml L^{-1}) mixed with polyoxyethylene-(3)-isotridecyl ether (TDE-3) (CAS 24938-91-8) (0.1 ml L^{-1}) as a spreader showed 90.1% and 64.3% control efficacy against *Aphis gossypii* Glover and *Myzus persicae* Sulzer (Homoptera: Aphididae) adults, respectively, at two days after the application in the greenhouse. Similarly, larger amounts of protein were detected in the supernatant precipitates after incubation with *A. gossypii* adults than *M. persicae* adults in the presence of TDE-3 from SDS-PAGE. Peculiarly, the incubations with TDE-3 made the bands stronger than those without TDE-3 in both aphid species.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-10

withdrawn

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-11

The biofumigant effects of *Muscodor albus* on potato tubermoth, *Phthorimaea operculella*, and codling moth, *Cydia pomonella.*

Lerry Lacey - USDA, Agricultural Research Service, Wapato, WA, USA
Address for correspondence: lerry.lacey@ars.usda.gov

The endophytic fungus, *Muscodor albus*, produces several volatile organic compounds (VOCs: alcohols, esters, ketones, acids and lipids) that are biocidal for a range of organisms. We conducted research on the insecticidal activity of *M. albus* VOCs on potato tubermoth (PTM) (*Phthorimaea operculella*) and codling moth (CM) (*Cydia pomonella*). The insecticidal activity of the fungus for control of PTM adults and neonate larvae was demonstrated after 78 hours of exposure under different temperature regimes and dosages of fungus. Adult PTM were very susceptible (91% mortality) to 30 g of hydrated fungal mycelium on rye seeds in a 28 liter chamber at 24°C. Neonate larvae under the same conditions responded with 73% mortality. Three day-old larvae within tubers were also susceptible but after longer exposures. A 7 day exposure to VOCs produced 96% mortality. VOCs were also tested against CM adults, neonate larvae, larvae in infested apples, and diapausing cocooned larvae. Fumigation of adult CM with VOCs for 78 hours resulted in 81% mortality. Exposure of neonate larvae to VOCs for 78 hours on apples and incubating for 7 days in fresh air resulted in 86% mortality. Exposure of apples that had been infested for 5 days, fumigated with VOCs for 78 hours, and incubated as above produced 71% mortality. Diapausing cocooned CM larvae that were exposed to VOCs for 7 or 14 days resulted in 31 and 100% mortality, respectively. Treating several stages of PTM and CM with VOCs indicate that M. albus could be an alternative to broad spectrum chemical fumigants.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-12

Monitoring on resistance of brown planthopper *(Nilaparvata lugens)* to imidacloprid and buprofezin

Jianhong Li - College of Plant Science and Technology, Wuhan, Hubei Province, People's Replublic of China; ***Liang Yao*** - College of Plant Science and Technology, Wuhan, Hubei Province, People's Replublic of China; ***Chunhua Qin*** - College of Plant Science and Technology, Wuhan, Hubei Province, People's Replublic of China; ***Wei Xu*** - General Station of Hubei Plant Protection, China, Wuhan, Hubei Province, People's Replublic of China; ***Kaixiong Zhang*** - General Station of Hubei Plant Protection, China, Wuhan, Hubei Province, People's Replublic of China; ***Chuanhua Peng*** - General Station of Hubei Plan Protection, China; ***Hangang Luo*** - General Station of Hubei Plan Protection, China,
Address for correspondence: jianhl@mail.hzau.edu.cn

In 2007, Seven field populations of *Nilaparvata lugens* were collected from Tongcheng, Wuxue, Tianmen, Jianli, Gongan, Xiaogan and Zaoyang respectively. The toxicity assay of imidacloprid and buprofezin to seven populations were done by rice stem-dipping method. The results showed that Tongcheng, Wuxue, Tianmen, Jianli, Gongan, Xiaogan and Zaoyang field populations produced high-level resistance to imidacloprid with resistance index varied from 69.00 to 153.33 compared with the susceptible colony. Xiaogan field population had high resistance to buprofezin with resistance index of 41.06, Tongcheng, Wuxue, Gongan, Jianli, Tianmen and Zaoyang field populations had middle-level resistance to buprofezin with resistance index varied from 13.39 to 23.40.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-13

Selection of Chilean entomopathogenic fungal strains to yellowjacket wasps.

Loreto Merino - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Andrés France*** - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Marcos Gerding*** - INIA Quilamapu, Chillán, Bio Bio, Chile
Address for correspondence: lmerino@inia.cl

The yellowjacket wasp, *Vespula germanica*, is considered a serious pest of productive and recreational activities worldwide. A pathogenicity study was carried out with a Chilean collection of 29 isolates of Metarhizium anisopliae and 30 of *Beauveria bassiana* against worker, male and queen wasps. Wasps of same age were fed with liquid sugar baits containing 1x108 conidia mL-1 suspensions of each isolate. Mortality was evaluated daily and the sporulation on the cadavers. The highest mortality and sporulation (p= 0.015) were obtained with isolates Qu-B941 and Qu-B933 of Beauveria bassiana, reaching 79 and 95% for workers, 66 and 73% for males and 63 and 81% for queens, respectively. A second experiment was accomplished with the isolates that reached percentile 90 of mortality and sporulation. Two isolates were provided at different concentration (0 to 1x108 conidia mL-1) by liquid bait to workers, then, mortality was evaluated daily. The results showed that 1 x 108 conidia mL-1 increased up to 90 and 97% workers mortality with Qu-B941 and Qu-B933 isolates, respectively.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-14

Conidial mass production of entomopathogenic fungi and tolerance of mass-produced conidia to UV-B radiation and heat.

Drauzio E. N. Rangel - Utah State University, Logan, UT, USA; ***Éverton K. K. Fernandes*** - Utah State University, Logan, UT, USA; ***Helen G. Bignayan*** - Bureau of Plant Industry, Philipinnes, Jordan, Guimaras, Philippines; ***Hernani G. Golez*** - Bureau of Plant Industry, Philipinnes, Jordan, Guimaras, Philippines; ***Donald W. Roberts*** - Utah State University, Logan, UT, USA
Address for correspondence: drauzio@biology.usu.edu

The most common systems worldwide for conidial production utilize sterile rice grains as the growth substrate. The current study investigated conidial mass production of six species of entomopathogenic fungi [*Metarhizium anisopliae* (*Ma*), *Beauveria bassiana* (*Bb*), *Aphanocladium album* (*Aa*), *Lecanicillium aphanocladii* (*La*), *Simplicillium lanosoniveum* (*Sl*) and *Verticillium lecanii* (*Vl*)] on white basmati rice or premium

short-grain brown rice in polypropylene bags. Four moisture conditions were compared: 1) 100% water (1:1 H_2O: rice by weight); 2) 100% water plus 5% peanut oil; 3) 100% water plus 10% peanut oil; 4) 75% water and 25% coconut milk. The bags were inoculated with suspensions of ~10^9 conidia of each isolate. The bags were incubated at 28 ± 1°C for 14 days. Conidia were then harvested and conidial concentrations determined by hemacytometer counts. The conidial viabilities were assessed on PDAY medium at 400µ magnification after incubation for 12h at 28°C. Aliquots of conidial suspensions were inoculated (without spreading) on PDAY + 0.002% benomyl in Petri plates and immediately exposed to 978 mW m^{-2} of Quaite-weighted UV-B for 2 h. Additionally, conidial suspensions were exposed to 45°C for 3 h, and 20-ml aliquots inoculated on PDAY + benomyl. The plates were incubated at 28°C, and germination assessed after 48h. Except for *La* (ARSEF 6433), conidial production generally was higher on white rice than on brown rice, regardless of moisture combinations. The 100% water condition provided higher conidial production for *Bb* isolates (ARSEF 252 and ARSEF 3462) and *Ma* (ARSEF 2341), while addition of 10% peanut oil enhanced conidial yield for *Sl* (ASEF 6430). Overall, conidia produced per gram of rice was high for *Bb, Sl, Vl* (ARSEF 6651) and *Aa* (ARSEF 1329), and low for *Ma* (ARSEF 1545 and ARSEF 2341) and *La*. The isolate with the highest conidial production (approximately $1.3µ10^{10}$ conidia g^{-1} of substrate) was *Bb* (ARSEF 3462) on white rice with 100% water. High conidial viability was observed, regardless of fungal isolate, type of rice, or moisture combinations. Viability of conidia grown on white rice with 100% water and stored at -20°C for 2 years decreased significantly for the *Ma* isolates, but not for *Vl, Sl,* and *Aa*. Conidia produced on rice with different moisture conditions did not differ in tolerance to UV-B radiation or heat. High tolerances to UV-B radiation and heat were observed for *Bb, Ma* and *Aa*, but low tolerances were observed for *La, Sl* and *Vl*. In fact, there was no germination of heat-treated conidia of the latter three species.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-15-STU

Evaluation on virulence of *Metarhizium* strains against green peach aphid *Myzus persicae.*

Le-tian Shan - Institute of Microbiology, Zhejiang University, Hangzhou, Zhejiang, P. R. China; ***Ming-guang Feng*** - Institute of Microbiology, Zhejiang University, Hangzhou, Zhejiang, P.R. China
Address for correspondence: hypodermic@yahoo.com.cn

Twenty-three isolates of *Metarhizium anisopliae*, *M. anisopliae* var. *anisopliae*, *M. anisopliae* var. *acridum*, *M. anisopliae* var. *majus* and *M. flavoviride* var. *minus* derived from different host insects worldwide were separately assayed for their pathogenicity and time-concentrationmortality (TCM) effect against *Myzus persicae* apterae in triplicate leaf-dish bioassays each consisting of the mean concentrations of 11.5, 99 and 1179 conidia mm^{-2} as fungal treatments plus blank control. All the tested isolates were proven pathogenic to *M. persicae* at 21°C and 14:10 L:D during 8-day observation and caused corrected mortalities of 0.8-95.3% at the high concentration despite aphid mycosis rarely attributed to *Metarhizium* spp. in nature. The TCM observations of 10 isolates causing >50% mortalities were well fitted to a TCM model, yielding parameters for estimating their time-depending LC 50 s and concentration-depending LT 50 s. Four isolates of *M. anisopliae* (ARSEF 759 and 576) and *M. anisopliae* var. *anisopliae* (ARSEF 4132 and 2080) were selected as excellent candidates for use in aphid control due to the smaller LC_{50}s of 44-80 conidia mm-2 on day 8, the shorter LT_{50}s of 4.9-6.8 days at 100 conidia mm^{-2}, and the high percentages of 91-98% mycotized among killed aphids. These indices highlight their high virulence to *M. persicae* and desired sporulation potential on cadavers. The biocontrol potential of other isolates with computable LC_{50} s and LT_{50} s is discussed with the percentages of mycotized cadavers.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-16-STU

Isolation and characterization of entomopathogenic fungi for the pine sawyer, *Monochamus saltuarius* Gebler.

Tae-Young Shin - Chungbuk National University, Cheongju, Chungbuk, Korea; ***Sung-Min Bae*** - Chungbuk National University; ***Jae-Bang Choi*** - Chungbuk National University; ***Hyun-Na Koo*** - Chungbuk National University; ***Soo-Dong Woo*** - Chungbuk National University, Cheongju, Chungbuk, Korea
Address for correspondence: sdwoo@cbnu.ac.kr

Pine wilt is the most important disease of pine trees in Korea, Japan and China. The pathogen causing this disease, the pinewood nematode (*Bursaphelenchus xylophylus*), is vectorially transmitted by adults of some Cerambycid beetle species, and the Japanese pine sawyer, *Monochamus alternatus*, is the major vector species in Korea. Although chemical insecticides have been used to kill the vector insect and thus prevent transmission of the pathogen, the efficacy is not good. In Japan, to control this insect, an entomopathogenic fungus was studied and developed as an insecticide. This is thought to be the convenient and effective method to control *M. alternatus.* Recently, there are several reports about the pinewood nematode being vectored by also the pine sawyer, *M. saltuarius,* in Korea. The objective of this study, therefore, was to isolate and identify entomopathogenic fungi from *M. saltuarius* cadavers to control it. We collected the cadaver of *M. saltuarius* and then screened several fungi colonies. The pathogenicity of each fungus was tested using oak longicorn beetle, as substitutive insect. *M. diphysis* is also serious pest to various trees in forest. As the result, only one of them showed high pathogenicity against *M. diphysis.* Selected fungus was identified by microscopic examination and DNA analysis. Pathogenicity was also evaluated to *M. saltuarius.*

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-17

Host specificity of microsporidia pathogenic to the gypsy moth, *Lymantria dispar* (l.): field studies in Slovakia.

Leellen Solter - Illinois Natural History Survey, University of Illinois, Champaign, IL, USA; ***Daniela Pilarska*** - Bulgarian Academy of Sciences, Institute of Zoology, Sofia, Bulgaria; ***Michael McManus*** - USDA Forest Service, Hamden, CT, USA; ***Milan Zubrik*** - Forest Research Institute, Banska Stiavnica, Slovakia; ***Jan Patocka (deceased)*** - Forest Research Institute, Banska Stiavnica, Slovakia; ***Wei-Fone Huang*** - Illinois Natural History Survey, University of Illinois; ***Julius Novotny*** - Forest Research Institute, Banska Stiavnica, Slovakia;
Address for correspondence: lsolter@illinois.edu

Eradication of the introduced gypsy moth, *Lymantria dispar* (L.), from North America was deemed impossible by the mid Twentieth Century, resulting in extensive evaluation and introduction of natural enemies from Europe and Asia for biological control of this serious defoliator. Of the numerous predators, parasites and pathogens studied, the *L. dispar* pathogens have proven to be the most valuable biological control agents. Of interest due to their ubiquity and diversity are the microsporidia; at least four species are important chronic pathogens of *L. dispar*. Never recovered from North American gypsy moth populations, the major issue regarding their introduction concerns safety to native non-target insects. In this study, we evaluated the effects of microsporidia on non-target Lepidoptera when microsporidia were released via ultra low volume sprays into field plots consisting of natural oak stands in

Central Slovakia. Coverage of infective spores in a complex environment and, thus, exposure of sympatric non-target species was maximized. We determined, based on host specificity, that three species of the microsporidia are appropriate organisms for release as classical biological control agents of *L. dispar*. We also evaluated risks that inundative release would pose to nontarget organisms and added to a basic understanding of microsporidian host range.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-18

On-farm control of the leaf miner, *Liriomyza trifolii* in cut flowers using *Isaria fumosorosea.*

Vitalis Wekesa - Indian River Research Center, University of Florida, IFAS, Fort Pierce, Florida, USA; ***Pasco Avery*** - Indian River Research Center, University of Florida, IFAS, Fort Pierce, Florida, USA; ***Cindy McKenzie*** - U.S. Horticultural Research Laboratory, ARS-USDA, Fort Pierce, Florida, USA; ***Charles Powell*** - Indian River Research Center, University of Florida, IFAS, Fort Pierce, Florida, USA; ***Lance Osborne*** - Mid-Florida Research and Education Center, University of Florida, IFAS, Apopka, Florida, USA
Address for correspondence: ewekesah@yahoo.com

The generalist entomopathogenic fungus, *Isaria fumosorosea* strain Apopka-97 (Wize) Brown and Smith (Hypocreales: Cordycipitaceae) has exhibited significant potential as a biological control agent of several important pests both in the field and greenhouse. Although this fungus has never been tested against *Liriomyza trifolii* (Burgess), its efficacy against other polyphagous pests and compatibility with other control measures makes it a good candidate for control of leaf miner flies. In this study, the relative effectiveness of the fungus, *I. fumosorosea* (PFR 97™) was compared to pesticides for the management of *L. trifolii* populations in *Gerbera* daisies and sunflower. The fungus was applied alone or in combination with pesticides (fungicides, insecticides and insect growth regulators) and the number of flies captured on yellow sticky cards as well as the number of leaf mines were compared with the untreated control blocks for four weeks. Very low numbers of adults were trapped in sunflower compared to the *Gerbera* daisies with all treatments being similar. In *Gerbera*, the number of flies significantly decreased with time in all the treatments compared to the untreated control, an indication that the population declined as a result of treatment application in this crop. However, there were no differences in the abundance of flies from 7 - 28 days post treatment between treatments, suggesting that PFR 97™ is as effective as any of the pesticide treatments. Results demonstrated that leaf miner control may not need joint application of this fungus with the pesticides hence reducing production costs.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-19

Evaluation of biological baits for paper wasp *Polistes dominulus* control.

Marcos Gerding - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Loreto Merino*** - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Andrés France*** - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Lindsay Barrios*** - INIA Quilamapu, Chillán, Bio Bio, Chile
Address for correspondence: lmerino@inia.cl

The paper wasp *Polistes dominulus* is a social invading wasp in Chile, particularly in urban places. The objective of this research was to evaluate two types of ingredients formulated in biological bait with conidia of *Beauveria bassiana* (isolate DW B933). Two baits were prepared on the base of honey or nutritive media (DIFCO®) by increasing the concentrations of each product. First, the conidia germination was evaluated during 30 days. The results indicate that as the honey concentration increase the conidial germination decrease. Instead, the nutrient media did not affect the germination at all the concentrations. Later, these baits were provided to worker wasp daily and for 10 days, in two conditions: with and without the presence of a nest. The bait consumption was evaluated daily. The results showed that the lonely wasp consume more honey. Otherwise, the wasps with its nest showed different nutritional requirements, since they preferred the consumption of nutritive media based baits.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-20

Evaluation of biological baits for paper wasp Polistes dominulus control.

Vladimir Gouli - University of Vermont, Burlington, Vermont, Chittenden; ***Margaret Skinner*** - University of Vermont, Burlington, Vermont, Chittenden; ***Bruce Parker*** - University of Vermont, Burlington, Vermont, Chittenden; ***Svetlana Gouli*** - University of Vermont, Burlington, Vermont, Chittenden; ***Cheryl Frank*** - University of Vermont, Burlington, Vermont, Chittenden
Address for correspondence: sgouli@uvm.edu

Survival of two entomopathogenic fungi, Beauveria bassiana (Bb), strain ERL-547 and Metarhizium anisopliae (Ma), strain ERL-607, was studied on marigold plants in the fall season. Fungi were applied as water suspensions of conidia (1 x 108 conidia per ml), and in a special experimental formulations including whey protein concentrate (WPC). Marigold plants were placed in a crop of ornamental bedding plants in a standard commercial plastic greenhouse. Leaf samples were collected after application and after 6, 12, 24 and 48 days. Adhesive tape prints were prepared from the upper and lower surfaces of the leaves to assess the number of viable and non-viable conidia. An imprint was incubated on potato dextrose agar (PDA), than prints were placed on glass slides and stained. Conidia viability before application was 98 ± 2% for Bb and 93 ± 4% for Ma, and immediately after spraying decreased by 13% and 37% for Bb and Ma, respectively. After 6 days for both formulations and strains >90% of the conidia had lost viability on the upper surface of the leaves; compared with 24-26% on the under sides of the leaves. The WPC formulated Bb conidia demonstrated a high level of viability after 6 days on the undersides of the leaves (over 75% viable conidia). The rate of conidial inactivation was significantly greater for the water than the WPC formulation. The Ma formulation demonstrated a considerably lower capacity for viability than the Bb formulations on the upper and lower leaf surfaces. Considering that most arthropod pests of greenhouse ornamentals are commonly found on the undersides of leaves, coverage to this area is critical for the effectiveness of treatments of entomopathogenic fungi. Based on these data, repeat applications of fungi must be made every two weeks to ensure necessary number of viable conidia on plant.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-21

Improved production of conidia *in vitro* by *Cordyceps militaris.*

In-Pyo Hong - National Academy of Agricultural Science, Kweonseon-gu Suwon, NY, Rep. of Korea; ***Richard A. Humber*** - USDA-ARS Biological IPM Research, Ithaca, NY, United States
Address for correspondence: richard.humber@ars.usda.gov

Two species entomopathogenic fungi most widely used and valued in traditional Asian medical practice are *Ophiocordyceps sinensis* (formerly *Cordyceps sinensis*) and *Cordyceps militaris*. Although *O. sinensis* may be the more famous and expensive fungus, it is also comparatively rare and cannot be grown or made to fruit readily in culture whereas *C. militaris*

occurs worldwide, grows readily in culture, and is the easiest of all *Cordyceps* species to fruit in culture. There is a well established cottage industry in Korea to produce *C. militaris* as a dietary supplement or even as a culinary ingredient used to promote improved health. Most of the Korean firms raising *C. militaris* obtain fruiting bodies from silkworms that are injected with suspensions of hyphal bodies grown in liquid cultures. This study seeks to facilitate and to simplify the infection process used to produce this fungus by finding a simple culture medium on which abundant supplies of the *Lecanicillium* conidial state of *C. militaris* are produced and can be used with simplified infection protocols involving spraying or dipping in conidial suspensions than the more material- and labor-intensive injection protocol. The studies to be reported include quantitative tests of conidial yields on varying carbon sources, varying nitrogen sources, and attempts to optimize the carbon/nitrogen ratio and pH of the medium for conidial production.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-22

Isolation of the cluster *thuabcdefg* for thuringiensin biosynthesis in *Bacillus thuringiensis* strain ct-43.

Xiaoyan Liu - Huazhong Agricultural University, Wuhan, Hubei, China; ***Lifang Ruan*** - Huazhong Agricultural University; ***Donghai Peng*** - Huazhong Agricultural University; ***ZIniu Yu*** - Huazhong Agricultural University; ***Ming Sun*** - Huazhong Agricultural University, Wuhan, Hubei, China
Address for correspondence: m98sun@mail.hzau.edu.cn

Construction of libraries has turned into an effective and necessary approach for genome-wide screens to find some new functional genes. Here we isolated the biosynthesis cluster of thuringiensin (?-exotoxin) by modification of such an approach. First, a bacterial artificial chromosome (BAC) shuttle library of *Bacillus thuringiensis* strain CT-43 was constructed with a *Bacillus thuringiensis-Escherichia coli* shuttle vector. Next we carried out the heterogenous expression works of those BAC clones in plasmid-cured *B. thuringiensis* BMB171 and obtained a clone BMB0542 which could produce much thuringiensin. Sequence analysis showed that pBMB0542 included an acyl carrier protein-dependent cluster about 12 kb namely thuABCDEFG. At the same time, with plasmids elimination assay and Southern blot, the thuringiensin biosynthesis cluster was located on a large plasmid harboring Cry1Ba gene named pBMB0558 (109,464bp) in Bacillus thuringiensis CT-43. Based on the sequence of pBMB0542 and Cry1Ba gene, the plasmid pBMB0558 was indirectly cloned and sequenced. According to these results, we deduced the thuringiensin biosynthesis pathway and identified it preliminarily by knocking out the ORFE in cluster thuABCDEFG, which had 31% identities to shikimate kinase in *Beijerinckia indica* subsp. *indica* ATCC 9039. LC-microTOF and LCMS-IT-TOF results proved the feasibility of this pathway and revealed that the biosythesis pathway of thuringiensin was mainly made of three processes: the biosynthesis of allose diacid, assembly of adenosine glucose allose diacid and the phosphorylation.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-23

Evaluating the potential use of an entomopathogenic fungus to control the *Cycad aulacaspis* scale in Florida.

Veronica Manrique - University of Florida, Ft. Pierce, Florida, USA; ***Jose Castillo- Altamirano*** - University of Florida, Ft. Pierce, Florida, USA; ***Pasco Avery*** - University of Florida, Ft. Pierce, Florida, USA; ***Ronald Cave*** - University of Florida, Ft. Pierce, Florida, USA
Address for correspondence: pbavery@ufl.edu

The cycad Aulacaspis scale (CAS) *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae), is a native insect in Southeast Asia, but it is a serious pest of ornamental cycads in Florida. Pest management is very difficult because expensive pesticide applications must be frequent and regular. The fungus *Isaria fumosorosea* Wize (=*Paecilomyces fumosoroseus*) is a geographically widespread entomopathogen of several insect orders, and is commonly found infecting whitefly nymphs and adults in the field. Two studies were conducted to evaluate the effectiveness of *I. fumosorosea* to infect CAS under laboratory and field conditions. Results from the laboratory showed that first and second instars of the CAS became infected with the fungus. High mortality was recorded with the high fungal dosage (1 g PFR 97™/100 ml distilled water) at 20 and 30° C (70-90% infection). In a field study, four cycad plants infested with CAS were randomly assigned to each of the following treatments in October 2008: 1) *I. fumosorosea* fungal application (1 g PFR 97™/100 ml distilled water) and 2) distilled water application (controls). Results showed that applications of *I. fumosorosea* significantly reduced numbers of second instar females of CAS compared to untreated plants. In addition, fungal infections of scales persisted in the field for 19 days in October-November 2008. These preliminary data suggest that field applications of *I. fumosorosea* could be used to reduce CAS populations in the field. However, field studies should be repeated during the summer-early fall when the environmental conditions are more favorable for both the scale and the fungus (higher temperatures and higher humidity).

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-24

Development and evaluation of *Isaria fumosorosea* for management of Asian citrus psyllid in Texas dooryard citrus.

Patrick J. Moran - USDA-ARS, BIRU, Weslaco, TX, USA; ***H. Enrique Cabanillas*** - USDA-ARS, BIRU, Weslaco, TX, USA; ***Mark A. Jackson*** - USDA-ARS, NCAUR, Peoria, IL, USA; ***Christopher A. Dunlap*** - USDA-ARS, NCAUR, Peoria, IL, USA; ***Pasco B. Avery*** - UF-IFAS Indian River Research and Education Center, Fort Pierce, FL, USA; ***Wayne B Hunter*** - USDA-ARS; ***David G. Hall*** - USDA-ARS,
Address for correspondence: humberto.cabanillas@ars.usda.gov

The Asian citrus psyllid *Diaphorina citri* Kuwayama vectors the causal agent of citrus greening disease or Huanglongbing (HLB), which has caused major economic losses in citrus in Florida and elsewhere in the world. The psyllid is present throughout the Lower Rio Grande Valley, the prime citrus-growing region of Texas, raising concerns that HLB will soon begin to affect citrus in the area. In this study, the fungus *Isaria fumosorosea* (formerly *Paeciliomyces fumosoroseus*, *Pfr*, ARSEF3581) is being evaluated for biological control of *D. citri* in ornamental and dooryard citrus, as part of an integrated strategy to manage Asian citrus psyllid in both production citrus and widespread residential plantings in Texas. Blastospores formulated in diatomaceous earth and other ingredients were resuspended and applied (2-4 x 10^7 spores per ml, 300 spores per mm surface area) to non-sticky yellow and green cards. Exposure of psyllids to fungus-coated cards in the laboratory led to 90% mortality within one week when psyllids were fed detached leaves of orange jasmine (*Murraya paniculata*) or whole sour orange plants (*Citrus aurantium* ssp. *aurantium*), but less than 50% mortality occurred when psyllids were fed whole orange jasmine plants with flushes. Behavioral observations and examinations of live and killed *D. citri* confirmed that psyllids acquired blastospores from *Pfr* 3581-coated cards and could infect other groups of psyllids that had not been exposed to cards. Field trials are determining blastospore acquisition and development of infected psyllid populations on sweet orange trees treated with fungal cards.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-25

Conidial pigmentation protects DNA from UB-B induced damage in the entomopathogenic fungus *Metarhizium anisopliae.*

Érika Nascimento - Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil; ***Everaldo Marques*** - Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil; ***Ludmilla Tonani*** - Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil; ***Donald Roberts*** - Utah State University, Logan, Utah, USA; ***Gilberto Braga*** - Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil
Address for correspondence: gbraga@fcfrp.usp.br

Conidia are responsible for the reproduction, dispersal and environmental persistence of many fungal species. In hyphomycetous pathogenic species like *Metarhizium anisopliae,* conidia frequently are the structures responsible for host infection. One of the main environmental factors that can kill and/or damage the conidia is solar UV radiation [UVA (320-400 nm) and UVB (290-320 nm)]. The cyclobutyl pyrimidine dimer is one of the major DNA photoproducts induced by UVB radiation. Precise quantification of this damage will be essential to better understanding of the mechanisms involved in conidial tolerance to solar radiation. The main goal of the present study was to quantify induction of pyrimidine dimers in DNA of both wild-type and albino conidia exposed to sub-lethal doses of UVB radiation. Conidia of the wild-type *M. anisopliae* var. *acridum* (ARSEF 324, a relatively UVB tolerant strain) were exposed to 1000 mW m-2 UVB irradiance for 15, 30, 60 and 90 min. Total Quaite-weighted doses were 0.9, 1.8, 3.6 and 5.4 kJ m-2, respectively. DNA was extracted from conidia immediately after the exposures and digested with T4 endonuclease V, which cuts one strand adjacent to the dimer. The fragments were resolved by quantitative alkaline gel electrophoresis. Densitometric analysis of the gels allowed the estimation of the median molecular length of the fragments from the different treatments. This information was used to estimate the number of dimers formed per 10 kb of DNA. DNA extracted from non-exposed conidia was used as control. The frequency of dimers was directly proportional to the dose, with 0.030, 0.057, 0.083 and 0.133 dimers / 10 kb DNA detected at the doses of 0.9, 1.8, 3.6 and 5.4 kJ m-2, respectively. With wild-type M. *anisopliae* var. *anisopliae* (ARSEF 23, a relatively UVB-susceptible strain) and an albino mutant of ARSEF 23 (ARSEF 6998), the number of dimers detected in the DNA of conidia exposed to the dose of 1.8 kJ m-2 was 0.276 / 10 kb DNA for the albino mutant. This was significantly higher than that observed in the wild-type ARSEF 23 (0.030), suggesting that green pigmentation may protect conidial DNA against the damage induced by UVB radiation.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-26

Survival of fungal isolates active against pear thrips in Vermont sugar maple forests.

Bruce Parker - University of Vermont, Burlington, Vermont, Chittenden; ***Svetlana Gouli*** - University of Vermont, Burlington, Vermont, Chittenden; ***Vladimir Gouli*** - University of Vermont, Burlington, Vermont, Chittenden; ***Margaret Skinner*** - University of Vermont, Burlington, Vermont, Chittenden; ***Jae Su Kim*** - University of Vermont, Burlington, Vermont, Chittenden
Address for correspondence: sgouli@uvm.edu

Periodic outbreaks of Pear thrips, *Taeniothrips inconsequens* cause significant foliage injury in the Northeastern US. Insects remain in the soil for 10 months of the year, and significant levels of natural mortality are caused by entomopathogenic fungi in the forest litter and upper part of the soil. Applications of active fungal propagules in the upper part of the forest soil can reduce the number of pear thrips in maple stands. We studied the survival of *Beauveria bassiana* (Bb), strains GHS and ERL-1170, and *Metarhizium anisopliae* (Ma), strains ERL-824 and ERL-601, in two formulations, millet- and starch-based granules. Before application, the millet-based formulation was mixed with pure millet at a 1:10 ratio to provide additional nutrient substrata for the fungi in the soil. The starch-based formulation was mixed with pure starch granules at the same ratio. Three forest stands predominating in sugar maple were selected at test sites; 33 dominant sugar maple trees were selected at treatment sites in each forest. An area of 1 x 1 m adjacent to each tree was designated as a plot. Soil samples for analysis were collected before application, after 1 hour and after 2, 4, 8, 12 and 16 weeks. Fungi were isolated by plating soil suspensions on selective media. After two weeks significant increase in the fungal population was found in the all experimental plots. This increased fungal population declined gradually after the peak at 2 weeks in three of the four test isolates. In contrast, for strain ERL-601 the number of fungal propagules continued to increase at 16 weeks. The survival of all of the fungal isolates was significantly better for the formulations from millet than starch. In general the Bb strains survived longer and at higher concentrations than the Ma strains.

Poster/Microbial Control - Monday, 16:30 - 17:30 MC-27

Efficient and eco-friendly recombinant baculovirus insecticide.

Hee Jin Shim - Department of Agricultural Biotechnology, Seoul National University, Seoul, Seoul, South Korea; ***Jae Young Choi*** - Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Seoul, South Korea; ***Yong Wang*** - Department of Agricultural Biotechnology, Seoul National University, Seoul, Seoul, South Korea; ***Jong Yul Roh*** - Department of Agricultural Biotechnology, Seoul National University, Seoul, Seoul, South Korea; ***Soo Dong Woo*** - College of Agriculture, Life and Environment Sciences, Chungbuk National University, Seoul, Seoul, South Korea; ***Byung Rae Jin*** - College of Natural Resources and Life Science, Dong-A University; ***Yeon Ho Je*** - Department of Agricultural Biotechnology, Seoul National University,
Address for correspondence: cjy1147@dreamwiz.com

A novel recombinant baculovirus, NeuroBactrus, was constructed to develop an improved baculovirus insecticide with additional beneficial properties such as higher insecticidal activity and recovery to wild-type baculovirus. For this, *Bacillus thuringiensis* crystal protein gene (cry1-5) was introduced into Autographa californica nucleopolyhedrovirus (AcMNPV) genome by fusion of polyhedrin-cry1-5-polyhedrin under the control of poyhedrin gene promoter. In the opposite direction of this fusion gene, an insect-specific neurotoxin gene (AaIT) under the control of early promoter from Cotesia plutellae bracovirus was introduced by fusion of orf603 partial fragment. Western hybridization and confocal microscopy revealed that AaIT neurotoxin and Polyhedrin-Cry1-5-Polyhedrin fusion protein expressed by the NeuroBactrus and that the fusion protein occluded into the polyhedra. In addition, the fusion protein was activated as about 65 kDa of crystal protein when treated with trypsin. The NeuroBactrus showed high level of insecticidal activity against *Plutella xylostella* larvae and significant reduction in median lethal time (LT_{50}) against *Spodoptera exigua* larvae compared to those of wild-type AcMNPV. Re-recombinants derived from the NeuroBactrus, NBt-Del5 (deleted cry1-5), NBt-DelA (deleted AaIT) and NBt-Del5A (deleted cry1-5 and AaIT; wild-type baculovirus) were generated in serial passages *in vitro* and *in vivo.* These results suggested that the NeuroBactrus could be transferred to wild-type baculovirus along with serial passages by the homologous recombination between two polyhedrin genes and two partial orf603 fragments.

NEMATODES

Poster/Nematodes . Monday, 16:30 - 17:30 N-1

Rhabditis (oscheius) species (Nematoda: Rhabditidae), associate with *Agrilus planipennis.*

George Kyei-Poku - Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada; ***Debbie Gauthier*** - Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada; ***Kirsty Wilson*** - Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada; ***Kees vanFrankenhuyzen*** - Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada; ***Johny Shajahan*** - Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada;
Address for correspondence: johny.shajahan@nrcan.gc.ca

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae) is an invasive pest ash trees, *Fraxinus* spp. It was discovered in 2002 in a small area in North America around the cities of Detroit Michigan, USA, and Windsor Ontario, Canada. Despite regulatory efforts to quarantine and eradicate EAB, this invasive beetle continues to spread throughout North America and has caused the death of millions of ash trees. While searching for natural enemies of EAB in Canada, we recovered a new nematode species from cadavers of this beetle. Molecular data (28S and internal transcribed spacer region (ITS) of rDNA sequences) and phylogenetic analyses demonstrated that the new species is closely related to other *Rhabditis* (*Oscheius*) species. A rare mating system, androdioecy (populations consisting of males and hermaphrodites) was observed in this new nematode species. Detail morphological characterization is on-going. The isolates kill EAB larvae and adults, *Tenebrio molitor* and *Zophobas morio* larvae within 2-4 days and have shown entomopathogenic ability to multiply on these coleopteran larvae. Bioefficacy studies against *Tenebrio molitor* EAB revealed that the new nematodes caused 92 and 98% mortality respectively in these beetles. Therefore this new nematode has the potential to be used as a biocontrol agent against EAB.

Poster/Nematodes - Monday, 16:30 - 17:30 N-2-STU

Survey of entomopathogens attacking larval western corn rootworm *Diabrotica virgifera virgifera* in Iowa cornfields.

Melissa Rynerson - Iowa State University, Ames, IA, USA; ***Aaron Gassmann*** - Iowa State University, Ames, IA, USA
Address for correspondence: rynerson@iastate.edu

Natural populations of entomopathogenic fungi and nematodes are ecologically important pathogens of soil-borne pests. The western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is a major soil-borne pest of corn *Zea mays* L. in both the United States and Europe, where larval feeding on corn roots can cause severe economic losses. We surveyed corn fields in Iowa, USA for naturally occurring populations of entomopathogenic fungi and nematodes. Five cornfields, which were widely spaced across the state and displayed high corn rootworm pressure during the summer, were sampled during early October 2008. Entomopathogens were recovered by baiting with three insect species: *D. v. virgifera, Tenebrio molitor* (Coleoptera: Tenebrionidae) and *Galleria mellonella* L (Lepidoptera: Pyralidae). The overall average mortality of each species was significantly different, with wax worms and mealworms having much higher mortality than the corn rootworm. Nematodes killed twice as many corn rootworm as fungi, and appear to be the more lethal pathogen to this soil-borne pest. We report a high prevalence of both fungi (*Beauveria bassiana* and *Metarhizium anisoplia*) and nematodes (*Steinernema spp.* and *Heterorhabditis spp.*) in cornfields, but only a subset of these pathogens appear to kill the western corn rootworm.

Poster/Nematodes - Monday, 16:30 - 17:30 N-3

Directional movement of entomopathogenic nematodes in response to electrical current.

David Shapiro-Ilan - USDA-ARS, SEFTNRL, Byron, GA, USA; ***James Campbell*** - USDA-ARS, GMPRC, Manhattan, KS, USA; ***Ed Lewis*** - UC Davis, Davis, CA, USA; ***Daniel Kim-Shapiro*** - Wake Forest University, Winston-Salem, NC, USA
Address for correspondence: David.Shapiro@ars.usda.gov

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are important regulating agents of insect populations. The infective juvenile nematodes respond to a variety of stimuli that aid in survival and host finding. Host finding strategies among entomopathogenic nematode species differ along a continuum from ambush (sit & wait) to cruiser (search & destroy). Identification of novel cues in the nematodes' environment can help us understand their basic ecology and enhance biocontrol efforts. We measured directional movement in response to an electrical current in five steinernematids (*S. carpocapsae, S. feltiae, S. glaseri, S. riobrave,* and *S. siamkayai*) and four heterorhabditids (*H. bacteriophora, H. indica, H. georgiana,* and *H. megidis*). The experiments were conducted in the laboratory. Electrical fields were generated on agar plates. None of the heterorhabditid species responded directionally to the electrical current except *H. georgiana,* which moved to a higher electrical potential. In contrast, all of the steinernematids responded directionally to the electrical field. Differential movement among the steinernematids species tended to reflect foraging strategy. Specifically, *Steinernema glaseri* (a cruiser) moved to a higher electric potential, whereas *S. carpocapsae* and *S. siamkayai,* (ambushers), moved to a lower electric potential. One intermediate forager, *S. riobrave,* moved to a higher potential, whereas the other intermediate forager, *S. feltiae,* moved to a lower potential. Thus, we hypothesize that entomopathogenic nematodes (primarily steinernematids) detect electrical currents or electromagnetic fields in nature, and these stimuli may be used differentially among species for host finding or enhancing other fitness characters.

Poster/Nematodes - Monday, 16:30 - 17:30 N-4

Pest and host plant effects of Ditera, a biopesticide used to control *Meloidogyne incognita.*

Kenneth Spence - University of California at Davis, Nematology, Davis, CA, USA; ***Ed Lewis*** - University of California at Davis, Nematology, Davis, CA, USA
Address for correspondence: kospence@ucdavis.edu

The materials that are designated as biopesticides may have complicated effects on the soil system. They may have direct lethal or sub-lethal effects on the nematode; have fertilizer effects on the plant which may offset nematode damage; and/or influence the interaction between the plant and the nematode, perhaps by inducing plant defenses. The present study examines the effect of DiTera®, a biopesticide derived from the soil fungus *Myrothecium verrucaria*, within the tomato-*Meloidogyne incognita* system. Laboratory assays suggested that DiTera may repel nematodes while stimulating local root proliferation, thus potentially providing plant roots with enemy free space. However, greenhouse studies provided little evidence that DiTera reduced nematode performance. Future work with field-soil may reveal a role for soil microbes in this interaction.

Poster/Nematodes - Monday, 16:30 - 17:30 N-5

Host range and production of *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) isolated from Ukraine.

Tatyana Stefanovska - National University of Life and Environmental Science, Kiev, Kiev, Ukraine; ***Harry K Kaya*** - University of California, Department of Nematology, Davis, CA, USA
Address for correspondence: steftat@hotmail.com

Studies were conducted in the laboratory to determine the host range, specificity and reproduction of a recently isolated *Heterorhabditis bacteriophora* strain ukr from Ukraine. The host range study included insect species from four orders: Lepidoptera (2 families), Coleoptera (5 families), Diptera (2 families), and Orthoptera (one family). The experiments were conducted by exposing 20 infective juveniles (IJs) of *H. bacteriophora* to each insect host. We found that *H. bacteriophora* infected insect species in the four orders with mortality ranging from 12% to 90%. The most susceptible hosts were lepidopteran and coleopteran species. This nematode species reproduced and completed its life cycle in insects of all four orders. We also demonstrated that *H. bacteriophora* had better infectivity and production in immature insect hosts compared to adults. In a separate study, *H. bacteriophora* caused 60% mortality at 5 IJs/*Galleria mellonella* (Lepidoptera: Pyralidae) larvae with a LD_{50} of 3 +-1 IJs/host. We also compared progeny production of *Steinernema carpocapsae* and *H. bacteriophora* ukr in two host insects, *G. mellonella* and *Tenebrio molitor* (Coleoptera: Tenebrionidae). Significance differences were observed in reproduction between the two nematode species in *T. molitor* and *G. mellonella.* Total progeny production of *S. carpocapsae* in *G. mellonella* averaged 158,000 IJs, whereas it averaged 132,300 IJs in *T. molitor.* Progeny production of *H. bacteriophora* in *G. mellonella* averaged 153,600 IJs, whereas it averaged 43,100 IJs in *T. molitor. Tenebrio molitor* is a suitable host for *S. carpocapsae* but not for *H. bacteriophora* ukr. The concentration of IJs per host influenced the progeny production with the optimal concentration ranging from 50 to 100 IJs/larva. Nematode progeny production in *T. molitor* was not related to the biomass of the cadaver.

Poster/Nematodes - Monday, 16:30 - 17:30 N-6-STU

Influence of *Steinernema carpocapsae* on the native nematode community in pistachio orchards.

Amanda K. Hodson - University of California-Davis, Davis, CA, USA; ***Edwin E. Lewis*** - University of California-Davis, Davis, CA, USA; ***Joel Siegel*** - USDA/ARS, SJVASC, Parlier, CA, USA
Address for correspondence: akhodson@ucdavis.edu

The entomopathogenic nematode, *Steinernema carpocapsae,* parasitizes the larvae of *Amyelosis transitella,* a key pest of pistachios. To achieve adequate biological control, *S. carpocapsae* are applied through the irrigation system at high densities. Their influence on native soil food webs remains unclear, particularly at large scales. The ecosystem to which *S. carpocapsae* are applied includes predatory, bacterivorous, fungivorous, and plant-parasitic nematode species. Reaching densities of 10 million per m^2, such native nematodes can serve as indicators of overall soil ecosystem diversity. *S. carpocapsae* may affect other nematodes through their mutualistic bacteria, which produce antimicrobial, nematicidal, fungicidal and insecticidal chemicals. If these chemicals repel certain nematodes, we would expect to see their abundance decrease in areas of application. This study quantifies *S. carpocapsae's* effects on native nematode diversity in two 40 acre orchards in Madera Co., California. *S. carpocapsae* were applied by micro-sprinkler to 35 trees in a randomized block design in March 2008. Adjacent trees were designated as controls using temporary irrigation plugs, and so received no nematodes. We compared nematode abundance and diversity in soil samples 2 days before and 1, 3, 5, and 10 weeks after application. Species abundances were used to calculate indices of ecological structure and nutrient enrichment. We repeated the experiment in a separate pistachio orchard in March 2009. In both 2008 and 2009, total nematode percent increase was reduced in *S. carpocapsae* treated trees compared with control trees after one week. *S. carpocapsae* may be repelling certain groups of nematodes thus decreasing the overall increase per tree. Changes in nematode diversity could indicate other non target effects on bacteria, fungi and nutrient availability, affecting soil health. Later samples are currently under analysis. 17:30 - 19:00

Microsporidia Division Workshop Monday, 20:00 - 21:00
Painted Horse I-II

Staining Techniques Used for Microsporidia Infecting Invertebrates

Organizer: Dorte Goertz

Workshop Paper - Monday, 20:00 35

Localisation of microsporidia in amphipod hosts using *in situ* hybridization.

Aurore Dubuffet - University of Leeds, Leeds, West Yorkshire, United Kingdom; ***Judith Smith*** - University of Leeds, Leeds, West Yorkshire, United Kingdom; ***Alison Dunn*** - University of Leeds, Leeds, West Yorkshire, United Kingdom
Address for correspondence: aurore_dubuffet@yahoo.fr

Many microorganisms are found in high densities in some tissues of their hosts while they are absent or only present at low densities in other tissues. This tissue tropism is generally related to their effects on their hosts and/or their transmission strategies. For example, symbiotic microorganisms which are beneficial to their hosts in terms of nutrition are generally observed in the gut, and vertically transmitted parasites are often seen in high densities in the gonads of their hosts. To study the tissue tropism of bacteria, fluorescent in situ hybridization (FISH) is widely used. However, this powerful technique has been neglected so far to study the distribution of microsporidia in their hosts, and electron microscopy is generally used instead. I will present the advantages and limits of FISH when applied to microsporidia, and will illustrate this technique (design of the probes & results) with our recent advances on the localization of 2 microsporidia, *Nosema granulosis* and *Dictyocoela duebenum* in the tissues of their host, the amphipod *Gammarus duebeni.*

Workshop paper - Monday, 20:20 36

Fluroescence staining for improved detection of microsporidian spores.

Andreas Linde - University of Applied Sciences, Eberswalde, Brandenburg, Germany; ***Thomas Kolling*** - University of Applied Sciences, Eberswalde, Brandenburg, Germany
Address for correspondence: alinde@fh-eberswalde.de

The detection of microsporidian spores in samples can be very difficult. They are easily visible for example in insect tissue smear preparations due to their birefringence when using phase contrast microscopy. In samples with low spore density or in stool samples, spores often go unnoticed, and phase contrast microscopy is not sufficient for differentiation. Therefore, the use of fluorescent stains has been introduced in 1982 by Vavra and Chalupsky. Stains like Uvitex B, Fluorescence Brightener 28 or Calcofluor White M2R react with the chitin in the endospore layer of microsporidian spores. These optical brighteners are organic compounds with aromatic rings which emit light in the visible spectrum upon UV or short-wave light excitation. Several modifications of this method

have been developed since then. For spore suspension, a solution of the brightener in phosphate buffer is recommended. Smear preparations are fixed with methanol or simply air dried and then flooded with the brightener. For better penetration of older or samples with higher densities, 1 N NaOH should replace the phosphate buffer. Bright fluorescent spores can be detected easily even in dense, contaminated samples after a few minutes of incubation time. The lowest concentration were 500 spores in 10ml of sample; 50 microscope fields had to be scanned to identify one spore. Optical brighteners can also be used on embedded (at least paraffin-embedded) tissue sections, at least when embedded in fixatives without picric acid. Commercial staining kits are also available. In this presentation, the methods will be described and compared, and results will be presented. Futhermore, ideas about other possible areas of application will be discussed.

Workshop paper - Monday, 20:40 37

Mitochondria related structures, staining of *Nosema* spp.

Yi-chun Tsai - National Taiwan University, Taipei, Taiwan, Taiwan; ***Chung-Hsiung Wang*** - National Taiwan University, Taipei, Taiwan, Taiwan
Address for correspondence: clementine_tsai@yahoo.com.tw

Microsporidia are long considered as amitocondrate organisms, however, some mitochondrial related proteins like mitochondrial Hsp70, pyruvate dehydrogenase, glycerol-3-phosphate dehydrogenase (mtG3PDH), and manganese-containing superoxide dismutase (MnSOD) were found in the microsporidia recently. Those finding catch the attention of researchers'. The incomplete components of mitochondria were called the "mitosome". An important question for now is where do those mitosome exist in the microsporidia? In our study, the micrisporidia isolated from yellow butterfly *Eurema blanda arsakia* (*Nosema* sp.) can be clearly stained by the mitochondrion specific dye. This method might be a convenient tool to study the mitosome or other organism in Microsporidia.

Virus Division Workshop Monday, 20:00 - 21:00
Arrowhead I-II

Advances in Invertebrate Cell Culture
Organizers: Just Vlak, Dwight Lynn

Workshop paper - Monday, 20:00 38

Tom Grace and his invaluable contribution to insect cell culture

Just Vlak - Department of Virology, Wageningen University, Wageningen, Netherlands
Address for correspondence: just.vlak@wur.nl

Tom Grace and his invaluable contribution to insect cell culture.

Workshop paper - Monday, 20:20 39

Use of cell cultures in the study of compounds with insect midgut action.

Guy Smagghe - Ghent University, B-9000 Ghent, Belgium
Address for correspondence: guy.smagghe@ugent.be

The insect midgut, the second largest organ after the skin, has been and is to date extensively studied for its central roles in the growth and physiology of insects and as a major target for novel biorational and biological insecticides like insecticidal crystals of *Bacillus thuringiensis* and insecticidal proteins like lectins and protease inhibitors. Studies on enterocytes with their typical brush border membrane and on stem cells, in the complex in vivo environment are simplified when moved to the in vitro situation. The culture described provides several unique features that permit studies not possible or difficult to do in vivo. As reported by Loeb and Hakim (2001) and Hakim et al. (2006) cultures were first successfully established in the 1990s. In these, a semi-stable balance was established between stem cell growth, differentiation and death of the mature cells. Initial studies focused on characterizing the cultures, particularly on defining the roles of growth and differentiation factors. Today these same cultures can be used to study the physiology of single cells. In this paper we will report on the goals to identify mechanisms of novel insecticide toxicity, and the mechanisms by which insects can develop insecticide resistance. (References: Loeb, M.J., Hakim, R.S. 2001. Insect midgut epithelium in vitro: An insect stem cell system. Journal of Insect Physiology, 42, 1103-1111; Hakim, R. S., Blackburn, M., Corti, P., Gelman, D., Goodman, C., Elsen, K., Loeb, M., Lynn, D., Smagghe, G. 2006. Growth and mitogenic effects of arylphorin in vivo and in vitro. Archives of Insect Biochemistry and Physiology, 64, 63-73). This work was supported by the Fund for Scientific Research (FWO-Vlaanderen, Belgium).

Workshop paper - Monday, 20:40 40

Replication of *Agrotis segetum* granulovirus in continuous insect cell lines.

Dwight E. Lynn - INSell Consulting, Newcastle, ME, USA; ***Jan W.M. van Lent*** - Laboratory of Virology, Wageningen University, Wageningen, The Netherlands; ***Monique M. van Oers*** - Laboratory of Virology, Wageningen University, Wageningen, The Netherlands; ***Just M. Vlak*** - Laboratory of Virology, Wageningen University, Wageningen, The Netherlands
Address for correspondence: insell@roadrunner.com

The *Agrotis ipsilon* cell lines, IPLB-AiE1611T and AiEd6T, were inoculated with occlusion-body derived virions (ODV) of the *A. segetum* granulovirus (AgseGV). The capsules were dissolved by treatment with 0.1M NaH_2 CO_3 /0.1M NaCl for 30 min. to release the occluded virions followed by neutralization with an acidic salt solution and inoculation onto cells in 24-well tissue culture plates. Within a few days, numerous cells in the culture developed cytopathic effects consisting of darkened nuclei and enlarged cells when examined with phase contrast or Nomarski microscopy optics. After one week dense particles were seen within the nuclei of infected cells and the infection extended in the culture. Both cell lines replicated AgseGV equally well. Transfer of filtered supernatant from the ODV-inoculated culture to fresh cells led to similar cytopathic effects in the new culture suggesting the production of infectious (budded) virus from the infected cells. Electron microscopy of cells inoculated with the 2nd passage virus showed typical granulovirus capsules in the nuclei of many cells and in great numbers. The capsules are being tested for in vivo infectivity in *A. segetum* larvae. This is the first successful replication of this virus in cell cultures and one of the few examples of *in vitro* replication and passaging of granuloviruses. It opens up the possibility to study the molecular genetics and functional biology of AgseGV in much detail and to engineer this virus for better insecticidal properties. This work was made possible through a visitors grant from the Wageningen Graduate School 'Production Ecology & Resource Conservation'

TUESDAY — 18 August

Plenary Symposium — Tuesday, 08:00 - 10:00
Kokopelli Ballroom II-III

The Host-Pathogen Dance: Interactions between Insect Hosts and Their Pathogens

Moderator: Rosalind James
Organizers: Rosalind James, Donald Roberts

Plenary Symposium - Tuesday, 08:00 — 41

Dancing with alternate partners: The evolution of virulence factors in insect pathogenic fungi

Michael Bidochka - Brock University, St. Catharines, ON, Canada; ***Nemat Keyhani*** - University of Florida, Gainesville, FL, USA
Address for correspondence: bidochka@brocku.ca

Metarhizium anisopliae and *Beauveria bassiana* are insect pathogenic fungi that are ubiquitous in soils. We have generally assumed that they have adapted as insect pathogens through their interactions with insects. Here we hypothesize that the insect pathogenic fungi *M. anisopliae* and *B. bassiana* are preadapted for at least one component of insect pathogenesis by their survival strategy in soil against phagocytic predatory amoeba. Microscopy of GFP-expressing *M. anisopliae* and *B. bassiana,* phagocytosis assays, and amoeba mortality assays showed that these insect pathogenic fungi are phagocytosed by the soil amoeba, *Acanthamoeba castellanii,* survive and grow within the amoeba resulting in ameoba death. We suggest that the fungal insect pathogens, *M. anisopliae* and *B. bassiana,* are preadapted for survival against insect phagocytic hemocytes by life in the soil and their avoidance of predatory amoeba may also explain their broad insect host ranges.

Plenary Symposium - Tuesday, 08:40 — 42

Poldnaviruses as symbionts and immunosuppressive pathogens of insects

Michael Strand - University of Georgia, Athens, GA, USA
Address for correspondence: mrstrand@uga.edu

Insects rely upon a well-coordinated innate immune system for protection against foreign invaders. Not surprisingly, pathogens and parasites have evolved a diversity of counterstrategies for suppressing host insect defenses. Among the most important natural enemies of insects are thousands of parasitoid wasp species that carry polydnaviruses (PDVs). PDVs persist as proviruses in the wasp and replicate asymptomatically in a region of the female reproductive tract. When a wasp oviposits into a host, she deposits eggs and a quantity of encapsidated virus. PDVs do not replicate in the wasp's host but expression of PDV-encoded genes cause physiological alterations that are essential for survival of the wasp's progeny. Thus, a mutualism exists between PDVs and wasps as viral transmission depends on parasitoid survival and parasitoid survival depends on infection of the wasp's host by the virus. PDVs are divided into two genera, bracoviruses (BVs) and ichnoviruses (IVs), on the basis of their association with wasps in the families Braconidae and Ichneumonidae. Genome analysis reveals important similarities in the organization of BVs and IVs but they share almost no sequence homology with one another suggesting their association with parasitoids arose independently. Physiological studies indicate that PDVs are essential for survival of parasitoids, because of their ability to immunosuppress the defense responses of hosts attacked by associated wasps. Functional analyses further indicate the majority of the virulence factors encoded by PDVs target the signaling pathways that regulate host immune effector responses rather than effector molecules themselves. Overall, recent results provide important insight into the evolution of polydnaviruses and also identify several molecules with immunosuppressive functions.

Plenary Symposium - Tuesday, 09:20 — 43

Bt resistance management mambos nos. 1 and 2.

Bruce Tabashnik - Department of Entomology, University of Arizona, ***David Crowder*** - Dept of Entomology, University of Arizona, ***Yves Carrière*** - Dept of Entomology, University of Arizona, ***Aaron Gassmann*** - Department of Entomology, Iowa State University, ***Luke Masson*** - Biotechnology Research Institute, National Research Council of Canada, ***Alejandra Bravo*** - Instituto de Biotecnologia, Universidad Nacional Autónoma de México, ***Mario Soberón*** - Instituto de Biotecnologia, Universidad Nacional Autónoma de México
Address for correspondence: brucet@ag.arizona

Evolution of insect resistance threatens the continued success of transgenic crops producing *Bacillus thuringiensis* (*Bt*) toxins that kill some key pests. First generation *Bt* crops are *Bt* corn and *Bt* cotton producing single Cry1 toxins for control of lepidopteran pests. The main approach for delaying insect resistance to these *Bt* crops is the refuge strategy, which requires refuges of host plants without *Bt* toxins near *Bt* crops to promote survival of susceptible pests. Monitoring data for first generation *Bt* crops suggest that refuges can delay insect resistance, especially when the resistance is inherited as a recessive trait and refuges are abundant. Although most pest populations have remained susceptible, field-evolved resistance has been reported to *Bt* cotton producing Cry1Ac by *Helicoverpa zea* in the southeastern US, *Bt* corn producing Cry1F by *Spodoptera frugiperda* in Puerto Rico, and *Bt* corn producing Cry1Ab by *Busseola fusca* in South Africa. To thwart pest resistance, some second generation transgenic crops produce two different *Bt* toxins targeting the same pest. Although this 'pyramid' strategy is expected to work best when no cross-resistance occurs between the two toxins, incorporating the potential effects of unexpected cross-resistance in resistance management plans may help to sustain the efficacy of pyramided *Bt* crops. Other approaches for countering resistance include use of *Bt* vegetative insecticidal proteins (Vips), RNA interference, and modified *Bt* toxins genetically engineered to kill pests resistant to native toxins. Knowledge of insect resistance to *Bt* crops can be used to minimize risks and enhance benefits for the future.

Symposium (Bacterial Division) — Tuesday, 10:30 - 12:10
Kokopelli Ballroom II-III

Bt Resistance in the Real World

Organizers/Moderators: Bill Moar, Neil Crickmore

Symposium - Tuesday, 10:30 — 44

What is resistance? Coming up with practical and realistic definitions of resistance to *Bt* crops

Timothy Dennehy - Monsanto Company, St. Louis, MO, USA; ***Graham Head*** - Monsanto Company, St. Louis, MO, USA
Address for correspondence: timothy.dennehy@monsanto.com

The term 'insect resistance' is widely recognized to describe heritable changes in insects that make them less affected by insecticides. However, more informative definitions of resistance have varied widely in their content. One extreme view is that resistance must be based on demonstrable failure of insecticides in the field. An opposite extreme defines resistance

as a 10-fold reduction in susceptibility of a field population, relative to a reference strain. Notwithstanding these definitional disparities, producers of Bt crops must continue to uphold commitments to proactively manage insect resistance, including meeting US-EPA imperatives to define triggers for remedial action in response to putative resistance events. We will describe a conceptual framework that promotes coexistence of both laboratory- and field-based definitions of resistance. Laboratory-based bioassays are essential for proactive resistance monitoring and for isolation and characterization of resistance, ideally before it impacts field performance. Equally essential are estimates of the impact that specific resistances have on pest survival under field conditions. Owing to negative practical ramifications for agricultural producers, Industry and Regulatory sectors, changes to resistance management plans should not be triggered by the detection of minor resistance genes. Such changes should be contemplated only after it has been demonstrated that the resistance has the potential to appreciably increase pest survival under treated field conditions. Therein, it is critical to contemplate what we cannot predict with accuracy regarding fieldexpression of resistance and the unknown impact of specific allele frequencies, resistance intensities, and fitness costs associated with resistance. Laboratory bioassays can both under-estimate or over-estimate resistance. Examples will be discussed in which major-gene resistance was isolated in the laboratory but did not impact field performance, even after many years of product use. All this underscores the critical feedback provided by routine resistance monitoring coupled with field performance evaluations. Critical issues and common mistakes in establishing and interpreting baseline susceptibility data will be summarized with relevant examples.

Symposium - Tuesday, 11:00 45

How to measure, monitor, and evaluate *Bt* resistance in corn insect pests

Blair Siegfried - University of Nebraska, Lincoln, NE, U.S.; ***Terence Spencer*** - University of Nebraska, Lincoln, NE, U.S.
Address for correspondence: bsiegfried1@unl.edu

Baseline susceptibilities of European corn borer and western corn rootworm have been determined for the major Bt toxins deployed in transgenic maize. In all cases, baseline assessments were initiated before commercial release of transgenic maize. Estimating the level of intra- and inter-population variation in susceptibility that is naturally present is prerequisite to detecting biologically important changes in susceptibility. For European corn borer, diagnostic concentrations derived from the baseline data were designated based on the upper end of the 95% confidence interval of the LC99. Annual assessments of susceptibility to the Cry1Ab and Cry1F toxin have been conducted using both diagnostic bioassays and complete dose response assessments. European corn borer populations appear to remain susceptible to both Bt proteins, although some populations have exhibited higher rates of survival than expected in diagnostic bioassays, and additional tests to confirm heritability and on-plant survival were conducted. These tests confirmed that relatively high levels of resistance to both Cry1Ab and Cry1F were evident based on diet bioassays and on-plant assays and that the strains derived from these field collections exhibit some capacity to develop on transgenic plants. However, there has been no indication of increased levels of resistance in subsequent years of testing in the same area where resistance was detected and no evidence of reduced product efficacy. In the case of western corn rootworms, diagnostic concentration bioassays are not practical because of limited sensitivity to the toxin and the large quantities of purified protein that would be necessary to establish a reliable diagnostic concentration. Limitations with regard to western corn rootworm monitoring and alternative methods for resistance detection will be discussed. One of the biggest sources of variation in bioassay results has been the need to change the source of toxins periodically. As a consequence, we have tested various quantitative techniques to measure toxin concentration and purity. Results indicate considerable variability in toxin potency and in accuracy of various quantitative techniques. These results suggest that standardized methods to insure toxin integrity are critical to monitoring efforts.

Symposium - Tuesday, 11:30 46

Delaying *Bt* resistance development in the field

William Moar - Monsanto Company, St. Louis, Missouri, USA
Address for correspondence: moarwil@auburn.edu

Resistance development to Bt crops by the target pest(s) is the primary concern with the widespread use of this technology. As a result, various strategies are being used to delay resistance development. This presentation will discuss the scientific basis and practical aspects for each strategy (not including monitoring) to delay Bt resistance development in the field such as refugia, high dose, the use of pyramided traits, and other more traditional IPM strategies such as various insecticides, biological and cultural control. The primary model used to discuss these strategies will be Bt cotton and the target lepidopteran pests.

Symposium (Fungus Division) Tuesday, 10:30 - 12:15
Painted Horse I-II

Fungi in Soil Habitats—Doing it in the Dirt

Organizer/Moderator: Stefan Jaronski

Symposium - Tuesday, 10:30 47

Distribution patterns of fungal entomopathogens in soil habitats

Nicolai V. Meyling - University of Copenhagen, Department of Agriculture and Ecology, Frederiksberg C, Denmark
Address for correspondence: nvm@life.ku.dk

Fungal entomopathogens are seemingly ubiquitous in soils. An increasing number of surveys around the world have shown that ascomycete fungi from the Hypocreales can be isolated from soils of many habitats. Based on morphological characteristics of the fungi some patterns of distribution have emerged. However, recent developments in molecular characterization of entomopathogenic fungi have revealed significant new insights into the distribution of genetic groups of some taxa, including *Beauveria* spp, and possible restrictions to certain habitats. I will present and discuss some of these distribution patterns and include new findings which indicate that some fungi are restricted to underground lifestyles while others cycle between below- and aboveground habitats within an agroecosystem.

Symposium - Tuesday, 11:00 48

It's a jungle out there! Abiotic and biotic factors affecting entomopathogenic fungi in the soil arena

Stefan Jaronski - USDA ARS, Sidney, MT, USA
Address for correspondence: stefan.jaronski@ars.usda.gov

One might conclude the soil is a more congenial arena for using entomopathogenic fungi (EPF) than the phylloplane. No ultraviolet light, no rainfall washing conidia from foliage, no rapid attenuation of conidial deposits by rapid plant canopy expansion. The soil is cool, damp and dark – perfect for EPF, yes?. In reality, "It's a jungle out there!"

wherein ignorance (of the many factors that affect both fungal efficacy and persistence) can tempt one into (fatal) bliss. This presentation will summarize what we do and do not know about factors that can affect the successful outcome of using fungal pest control agents against soil insects. The effect of some factors are better known (e.g., microbial soil flora), or more obvious (e.g., temperature). Other factors are still poorly understood. One case in point is the interaction between soil texture, moisture, and insect behavior on the infectivity of individual conidia in soil. Soil texture can significantly affect the infectivity of conidia, as can the moisture content of the soil. But life is not that simple. In the case of larval *Diabrotica* increasing soil moisture decreases efficacy of *Beauveria* conidia. In the case of sugarbeet root maggot and *Metarhizium*, the reverse seems to be true. Use of granules can change the rules of the game substantially. While recent data indicate *Metarhizium* may colonize the rhizosphere, this phenomenon may not be as common as we think and the rhizosphere contains many microbes that can be antagonists of the EPF. Little is known about these microbe-fungus interactions. Very little is known about the interaction between soil macrofauna and EPF conidia. Collembola may not be susceptible to EPF but can they affect conidial titers as strongly as they do plant pathogenic fungi? What about mites and other fauna? What about soil-dwelling Protista?

Symposium - Tuesday, 11:30 49

Efficacy vs. soil insects: tales from the battlefront (successes, failures, & thoughts about why)

Jarrod Leland - Novozymes Biologicals, Salem, VA, US; ***Stefan Jaronski*** - USDA-ARS, Sidney, MT, US; ***Denny Bruck*** - USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR,
Address for correspondence: JRRL@novozymes.com

Entomopathogenic fungi may provide persistent control in soil environments but strategies for efficiently targeting insect pests must be considered. Research with various formulations of *Metarhizium anisopliae* and *Beuveria bassiana* in artificial and agricultural soils has helped to define these strategies but there is still room for improvement particularly in open field soils. Examples of field results against such targets as vine weevils, wireworms, root maggots, and thrips have demonstrated potential of entomopathogenic fungi for control of soil insect pests and identified future directions for improving efficacy. Application to artificial soil can allow for thorough distribution of spores resulting in consistent high levels of control. Penetration of spores into agricultural soils or through turf can be difficult requiring careful timing of application to coincide with the insect's lifecycle that move through a treated barrier or cultural practices that allow incorporation. A greater understanding of the impacts of soil environments on spore persistence and activity as well as interactions with the rhizosphere may further identify opportunities for improving product efficacy.

Symposium - Tuesday, 12:00 50-STU

The occurrence of entomopathogenic fungi from South African citrus soils

Tarryn Anne Goble - Rhodes University, South Africa, Grahamstown, Eastern Cape, South Africa; ***Joanna Dames*** - Rhodes University, South Africa, Grahamstown, Eastern Cape, South Africa; ***Martin Hill*** - Rhodes University, South Africa, Grahamstown, Eastern Cape, South Africa
Address for correspondence: g04g4915@campus.ru.ac.za

A survey on the occurrence of entomopathogenic (EP) fungi was undertaken on soils from citrus orchards and adjacent orchard margins (refugia) on conventionally and organically managed farms in the Eastern Cape Province in South Africa. An adapted method for baiting soil samples with key citrus pest, *Thaumatotibia leucotreta* (Meyrick) and *Ceratitis capitata* (Wiedemann) larvae, as well as with the standard bait insect, *Galleria mellonella* (Linn), was implemented. Sixty-two potentially useful EP fungal isolates belonging to 4 genera were collected from 288 soil samples, an occurrence frequency of 21.53%. The most frequently isolated EP fungal species was *Beauveria bassiana* (15.63%) which was recovered considerably more often than *Metarhizium anisopliae* var. *anisopliae*, (3.82%) or *Lucanicillium psalliotae* (1.39%). *Metarhizium flavoviride* (0.35%) and *Conidiobolus coronatus* (0.35%) were less frequently encountered. *Galleria mellonella* was the most effective bait insect used to isolate EP fungal species, as significantly more isolates were recovered (45 isolates) from this species than either *C. capitata* (11 isolates) or *T. leucotreta* (6 isolates). This study showed a significantly higher occurrence of EP fungi in soil samples taken from refugia compared to cultivated orchards of both organically and conventionally managed farms. However no significant differences were observed in the recovery of fungal isolates when both farming systems were compared. The isolation of B. bassiana from organically managed soils was significant when compared to its isolation from conventionally managed soils. In contrast, *M. anisopliae* var. *anisopliae* was isolated more frequently from soils of conventionally, cultivated soils than organically farmed soil samples but this result was not significant.

CONTRIBUTED PAPERS Tuesday, 10:30 - 11:45
White Pine I-II

Virus I

Moderators: Kelli Hoover, Peter Krell

Tuesday, 10:30 51

Viral-encoded fibroblast growth factor enhances virus spread via protease-mediated remodeling of basal lamina

John Means - Kansas State University, Manhattan, KS, U.S.A.; ***A. Lorena Passarelli*** - Kansas State University, Manhattan, KS, U.S.A.
Address for correspondence: lpassar@ksu.edu

Fibroblast growth factors (FGFs) are a family of growth factors involved in organogenesis, tumorigenesis, and wound repair and are encoded by both vertebrates and invertebrates. Baculoviruses are the only known viruses that encode an *fgf* homolog (*vfgf*). Mutants of *Autographa californica* M nucleopolyhedrovirus (Ac*M*NPV) lacking *vfgf* exhibit delayed infection of several host tissues and delayed host mortality following oral infections. We previously compared morphological changes that occurred in the basal lamina of epithelial cells infected with viruses carrying or lacking *vfgf* and observed remodeling of basal lamina components in the presence of *vfgf*. In this study we identified the protease cascade responsible for cleavage of basal lamina proteins and show that inhibition of the identified proteases hinders basal lamina rearrangement and virus spread.

Contributed Paper - Tuesday, 10:45 52

Targeted immunosuppression: a tool to study the host response to baculovirus infection

Nor Chejanovsky - Agricultural Research Organization, Bet Dagan, Israel; ***Hadassah Rivkin*** - Agricultural research Organization, Bet Dagan, Israel; ***Bruce A. Webb*** - University of Kentucky, Lexington, USA; ***Sassan Asgari*** - School of Integrative Biology, St. Lucia,

Queensland, Australia
Address for correspondence: ninar@volcani .agri.gov.il

We developed a model system based on the Mediterranean pest *Spodoptera littoralis* that is highly resistant to infection with the *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) to study the immune reponse of Lepidopterans to baculovirus infection. AcMNPV infection of *S. littoralis* larvae induced melanization and encapsulation of the virus and apoptosis of hemocytes. For the above purpose we constructed various orally-infectious recombinant baculoviruses expressing polydnavirus genes that target the insect immune system at various levels, namely AcTV3 encoding Vn50 a serine proteinase homologue containing an amino-terminal clip domain, vPvank1 and vPvank3 encoding Pvank1 and Pvank3 homologues of inhibitor of immune signaling kappa beta proteins (IkB) and Hv1.1 encoding a cys-motif protein. gfp-mediated tagging of the recombinant viruses were used to follow the viral path in the host and biological assays showed significant improvement of the viral efficacy compared to wild type AcMNPV. These tools enabled us to delineate a primary pattern of the immune response of *S. littoralis* to infection with baculoviruses.

Contributed Paper - Tuesday, 11:00 53

Establishment of an insect cell clone that harbors a partial baculoviral genome and is resistant to homologous virus infection.

Qingbei Weng - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, Guangdong Province, P.R. China; ***Kai Yang*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, Guangdong Province, P.R. China; ***Wei Xiao*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, Guangdong Province, P.R. China; ***Meijin Yuan*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, Guangdong Province, P.R. China; ***Yi Pang*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, Guangdong Province, P.R. China
Address for correspondence: yangkai@mail.sysu.edu.cn

After serially undiluted passaging of Spodoptera exigua(SeMNPV) in Se301 cells, a persistent infection was established and a cell strain, in which no polyhedra or viral particles were observed, was cloned and designated as P8-Se301-C1. The P8-Se301-C1 cells were morphology similarly to, but grow slower than the Se301 cells. Moreover, upon infection with SeMNPV, progeny virus yields including budded viruses and polyhedra were significantly decreased in the P8-Se301-C1 cells. This suggests the P8-Se301-C1 cells possess the capacity of homologous interference. $TCID_{50}$ assay and ICA assay demonstrate no infectious virus was produced in the P8-Se301-C1 cells. PCR analysis shows that the viral ie-0 and polh genes were present in the cells, but dnapol and orf67 (ac38K) genes were absent, suggesting the P8-Se301-C1 cells harbor incomplete SeMNPV genomes. Dot blot analysis demonstrates that 0.32±0.16 ng of viral DNA content present in 1.25µ105 P8?Se301?C1 cells. Q-PCR assay shows the copy numbers of polh in the P8-Se301-C1 cells was significantly higher than in the Se301 cells. Nested RT-PCR demonstrates that polh transcripts presented in the P8-Se301-C1 cells. The facts that the P8-Se301-C1 cells carry low level partial viral genome, but no viral progenies were produced suggest a latent-like viral infection is present in the P8-Se301-C1 cells.

Contributed Paper - Tuesday, 11:15 54-STU

Novel *cpgv* isolates: deciphering the molecular mechanism involved in overcoming *cpgv* resistance in the codling moth

Karolin Eberle - DLR Rheinpfalz, Neustadt, Rheinland-Pfalz, Germany; ***Johannes A. Jehle*** - DLR Rheinpfalz, Neustadt, Rheinland-Pfalz, Germany
Address for correspondence: karolin.eberle@dlr.rlp.de

Resistance of codling moth against *Cydia pomonella* granulovirus (CpGV) has recently emerged in European orchards. The resistance allele is dominantly inherited on sex chromosome Z. Early infection events and CpGV DNA replication are blocked in resistant larvae. Recently, several CpGV isolates (CpGV-I12, -S, -R1, MadexPlus) have been found to overcome resistance to CpGV based biocontrol agents. To understand this ability, it is crucial to determine which genetic factor(s) are responsible. Therefore, the genomes of two different resistance overcoming isolates CpGV-I12 and -S were completely sequenced. Gene content was compared to the published sequence of CpGV-M1 as well as to the completely re-sequenced original isolate CpGV-M. Comparison revealed only one common alteration in the two resistance overcoming isolates. A stretch of 24nt present in the *pe38*-gene of CpGV-M was not present in CpGV-I12 and -S. The same mutation was found in other resistance overcoming isolates. To verify the potential role of a *pe38* mutation in approving or overcoming resistance, *pe38* was knocked out from a CpGV-M-bacmid. The *pe38*-gene of CpGV-I12 was transferred by homologous recombination into the CpGV-M-*pe38*-knock-out bacmid. A rescue-bacmid was constructed as a control. The different constructs were tested for infectivity by injecting DNA into resistant and susceptible larvae.

Contributed Paper - Tuesday, 11:30 55

The role of the *PI3K-Akt* signal transduction pathway in AcMNPV infection of *Spodoptera frugiperda* cells.

Wei Xiao - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, GuangDong Province, P. R. China; ***Yi Yang*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, GuangDong Province, P. R. China; ***Qingbei Weng*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, GuangDong Province, P. R. China; ***Kai Yang*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, GuangDong Province, P. R. China; ***Yi Pang*** - State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, GuangDong Province, P. R. China
Address for correspondence: yangkai@mail.sysu.edu.cn

Many viruses activate the phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway, thereby modulating diverse downstream signaling pathways associated with antiapoptosis, proliferation, cell cycling, protein synthesis and glucose metabolism, in order to augment their replication. To date, the role of the PI3K-Akt pathway in baculovirus replication has not been defined. In the present study, we demonstrate that infection of Sf9 cells with *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) elevated cellular Akt phosphorylation at 1 h post-infection. The maximum Akt phosphorylation occurred at 6 h post-infection and remained unchanged until 18 h post-infection. The PI3K-specific inhibitor, LY294002, suppressed Akt phosphorylation in a dose-dependent manner, suggesting that AcMNPV-induced Akt phosphorylation is PI3K-dependent. The inhibition of PI3K-Akt activation by LY294002 significantly reduced the viral yield, including a reduction in budded viruses and occlusion bodies. The virus production was reduced only when the inhibitor was added within 24 h of infection, implying that activation of PI3K occurred early in infection. Correspondingly, both viral DNA replication and late and very late viral protein expression were impaired by LY294002 treatment; LY294002 had no effect on immediate early and early-late protein expression. These

results demonstrate that the PI3K-Akt pathway is required for efficient baculovirus replication.

Contributed Paper - Tuesday, 11:45 56-STU

Cellular secretion induced by ACMNPV replication alters permissibility of cells for narrow host-range baculovirus replication

Xin-Hua Cheng - Miami University, Oxford, Ohio, USA; ***Lihua Wang*** - Miami University, Oxford, Ohio, USA; ***Tamer Salem*** - Miami University, Oxford, Ohio, USA; ***Xiao-Wen Cheng*** - Miami University, Oxford, Ohio, USA
Address for correspondence: chengx2@muohio.edu

AcMNPV has a wide host-range, infecting Sf21 and High 5 cells very well. *Thysanoplusia orichalcea* MNPV (ThorMNPV) can infect High 5 cells very well, but not Sf21 cells. An AcMNPV polyhedrin promoter-GFP expression cassette was inserted in the ThorMNPV genome to generate vThGFP. An AcMNPV (vAcRFP) expressing red fluorescent protein (RFP) gene was also constructed. Co-infection of Sf21 cells with vThGFP/vAcRFP showed enhanced vThGFP replication in Sf21. When media of Sf21 co-infected with vThGFP/vAcRFP was used for plaque assays in Sf21, the vThGFP plaque sizes were negatively correlated with the distances to the vAcRFP plaques. Conditioned media (CM) of Sf21 cells infected with vAcRFP after centrifugation to remove viruses enhanced vThGFP replication in Sf21 cells. When the CM was stored at 4 oC for over a month, the enhancing ability decreased and the enhancing ability disappeared after a year of storage. The CM was not heat-stable above 50 oC. However, CM from High5 cells infected by vAcRFP inhibited vThGFP replication. All these suggest that vAcRFP may stimulate the Sf21 cells or High5 cells to secrete peptides which serve as ligands to bind to the cells and trigger signal transduction to regulate vThGFP replication in Sf21 cells.

WEDNESDAY — 19 August

Symposium (Virus Division) Wednesday, 08:00 - 10:00
Kokopelli Ballroom II

Invertebrate Antiviral Response

Organizer/Moderator: Nor Chejanovsky

Symposium - Wednesday, 08:00 57

Mechanism of RNAi-mediated viral immunity in plants and invertebrates

Shou-Wei Ding - Department of Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA, US
Address for correspondence: dingsw@ucr.edu

My lab has been taking a comparative approach to study the RNAi-mediated host immune responses to RNA viruses in plants and in invertebrates. In this viral immunity, plants and invertebrates produce virus-derived siRNAs during infection to direct viral immunity by RNA interference. I shall present our recent findings on the mechanism and evolutionary conservation of the RNAi-mediated viral immunity in *Drosophila melanogaster*, *Caenorhabditis elegans* and *Arabidopsis thaliana*.

Symposium - Wednesday, 08:30 58

Dicer-2* mediated antiviral response in *Drosophila

Jean-Luc Imler - CNRS-UPR9022, Strasbourg, Alsace, France; ***Safia Deddouche*** - CNRS-UPR9022, Strasbourg, Alsace, France; ***Cordula Kemp*** - CNRS-UPR9022, Strasbourg, Alsace, France; ***Stefanie Muller*** - CNRS-UPR9022, Strasbourg, Alsace, France; ***Aidan Budd*** - EMBL, Heidelberg, Alsace, Germany
Address for correspondence: JL.Imler@unistra.fr

RNA interference is a major antiviral defense in plants and invertebrates. By contrast, vertebrates rely mainly on the inducible production of antiviral molecules by cytokines of the interferon family to fight viral infections. We have shown that infection of *Drosophila* with RNA viruses also triggers a strong inducible response. One of the genes induced, Vago, controls the viral load in the tissue in which it is induced. Induction of Vago by virus infection is blocked in flies expressing the viral dsRNA binding protein B2, a potent suppressor of RNAi, suggesting that sensing of viral dsRNAs triggers the inducible response in drosophila. Furthermore, induction of Vago by the picorna-like virus DCV is strongly impaired in Dicer-2 mutant flies. Induction of Vago however does not depend on other genes of the RNAi pathway, such as Argonaute 2 or r2d2, suggesting that Dicer-2 has a dual function in virus infected cells, regulating both RNAi and an inducible response. Interestingly, Dicer-2 shares an evolutionary conserved DExD/H box helicase domain with receptors of the RIG-I-like receptor family, which sense viral RNAs in mammalian cells and trigger expression of interferons. We demonstrate that point mutations in the DExD/H box helicase domain of Dicer-2 abolish induction of Vago expression by DCV, thus establishing a parallel between the sensing of viral RNAs leading to induced antiviral responses in flies and mammals.

Symposium - Wednesday, 09:00 59

Alphavirus-derived small RNAs modulate pathogenesis in disease vector mosquitoes

Kevin Myles - Virginia Tech, Blacksburg, VA, USA; ***Michael Wiley*** - Virginia Tech, Blacksburg, VA, USA; ***Elaine Morazzani*** - Virginia Tech, Blacksburg, VA, USA; ***Zach Adelman*** - Virginia Tech, Blacksburg, VA, USA
Address for correspondence: mylesk@vt.edu

Mosquito-borne viruses cause significant levels of morbidity and mortality in humans and domesticated animals. Maintenance of mosquito-borne viruses in nature requires a biological transmission cycle that involves alternating virus replication in a susceptible vertebrate and mosquito host. Although the vertebrate infection is acute and often associated with disease, continual transmission of these viruses in nature depends on the establishment of a persistent, nonpathogenic infection in the mosquito vector. An antiviral RNAi response has been shown to limit the replication of RNA viruses in flies. However, the importance of the RNAi pathway as an antiviral defense in mammals is unclear. Differences in the immune responses of mammals and mosquitoes may explain why these viruses are not generally associated with pathology in the invertebrate host. We identified virus-derived small interfering RNAs (viRNAs), 21 nt in length, in *Aedes aegypti* infected with the mosquito-borne virus, Sindbis (SINV). viRNAs had an asymmetric distribution that spanned the length of the SINV genome. To determine the role of viRNAs in controlling pathogenic potential, mosquitoes were infected with recombinant alphaviruses expressing suppressors of RNA silencing. Decreased survival was observed in mosquitoes in which the accumulation of viRNAs was suppressed. These results suggest that an exogenous siRNA pathway is essential to the survival of mosquitoes infected with alphaviruses and, thus, the maintenance of these viruses in nature.

Symposium - Wednesday, 09:30 60

Wolbachia-mediated antiviral protection in insects

Karyn Johnson - School of Biological Sciences, Brisbane, Queensland, Australia
Address for correspondence: karynj@uq.edu.au

Drosophila is an important model system for understanding interactions between invertebrates and viruses. Recent studies have shown that cellular antiviral immune responses include both the instrinsic RNA interference pathway and inducible responses that are controlled by immune pathway cascades involving Toll and Jak/STAT. In addition to these host responses, we demonstrated that that a bacterium protects *Drosophila melanogaster* from pathogenic viruses. In the presence of the maternally inherited symbiotic bacterium *Wolbachia*, Drosophila C virus (DCV) particles accumulate more slowly and virus induced mortality is substantially delayed. Flies are also protected from pathogenic effects of other RNA viruses including Flock House virus, and cricket paralysis virus. Remarkably, the extent of antiviral protection mediated by *Wolbachia* is similar that provided by the host antiviral responses, indicating that the interaction between *Wolbachia* and virus has important biological implications. *Wolbachia* are found in an estimated two-thirds of insect species, so if generalised, the antiviral protection may impact on both beneficial and pest insects, including insects that vector important viral diseases of humans, animals and plants. Understanding the mechanism by which *Wolbachia* protects flies will contribute significantly to our fundamental understanding of the natural processes that protect insects from the impact of virus infection.

CONTRIBUTED PAPERS Wednesday, 08:00 - 10:00
White Pine I-II

Bacteria II

Moderator: David Pauron

Contributed Paper - Wednesday, 08:00 61

withdrawn

Contributed Paper - Wednesday, 08:00 62-STU

An alfa-amylase is a receptor for *Bacillus thuringiensis* Cry4ba toxin in the malaria vector mosquito *Anopheles albimanus*

Maria Teresa Fernandez-Luna - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Sarjeet Gill*** - University of California, Riverside, Riverside, California, USA; ***Alejandra Bravo*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Mario Soberón*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Juan Miranda-Rios*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico
Address for correspondence: luna@ibt.unam.mx

Mosquitoes of the genus *Aedes, Anopheles* and *Culex* are serious human disease vectors for dengue, malaria and filarial parasites, respectively. As these vectors are prone to develop resistance to chemical insecticides, new forms of management are needed. It has been shown previously that Cry toxins of *Bacillus thuringiensis* subs. *israelensis* (Bti) are highly toxic to larvae mosquito. In this work we analyzed the toxicity of isolated mosquitocidal Cry proteins to *Anopheles albimanus,* the main vector for transmission of malaria in Mexico. We found that Cry4Ba and Cry11Aa of Bti are toxic to *An albimanus* larvae. Ligand blot binding analysis indicated that a 70 kDa glycosylphosphatidyl inositol-anchored protein present in midgut brush border membrane vesicles bound Cry4Ba and Cry11Aa. This protein was identified as an alfa-amylase by mass spectrometry and enzymatic activity assays. The alfa-amylase gene was cloned by means of 5´and 3´ RACE experiments. The recombinant alfa-amylase produced in E. coli was shown to specifically bind Cry4Ba and Cry11Aa toxins.

Contributed Paper - Wednesday, 08:15 63

Aedes aegypti cadherin serves as a putative receptor of the Cry11aa toxin from *Bacillus thuringiensis* subsp. *sections*

Jianwu Chen - Department of Cell Biology and Neuroscience, University of California, Riverside, CA, USA; ***Karlygash Aimanova*** - Department of Cell Biology and Neuroscience, University of California, Riverside, CA, USA; ***Claudia Martinez*** - Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico; ***Mario Soberón*** - Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico; ***Sarjeet Gill*** - Department of Cell Biology and Neuroscience, University of California, Riverside, CA, USA
Address for correspondence: jwchen@ucr.edu

The Cry11Aa toxin of *Bacillus thuringiensis* subsp. *israelensis* is most active toxin to *Aedes aegypti* in this strain. We previously reported (1) that in addition to a 65 kDa GPI-anchored alkaline phosphatase (ALP) the toxin also binds a 250 kDa membrane protein. Since this protein is of the size of cadherin, which in lepidopteran insects is an important Cry toxin receptor, we developed an anti-*Aedes* cadherin antibody. This antibody was able to detect a 250-kDa protein in immunoblots of the brush border membrane vesicles (BBMV) prepared from larval guts. In addition the antibody inhibits Cry11Aa toxin binding to BBMV and immunolocalizes the cadherin protein to apical membranes of distal and proximal caecae and posterior midgut epithelial cells. This localization is consistent with areas to which Cry11Aa toxin binds and causes pathogenicity. Therefore, the full-length *Aedes* cadherin cDNA was isolated from *Ae. aegypti* larvae and partial overlapping fragments that covered the entire protein were made and expressed in *E. coli.* The cadherin ectodomain contains 11 cadherin repeats (CR), a transmembrane domain and intracellular domain. Using toxin overlay assays we showed that one cadherin fragment that contains CR 7-11 bound Cry11Aa. The Cry11Aa toxin bound the cadherin fragment with high affinity with an apparent Kd of 16.7 nM. These results indicated that Aedes cadherin is possibly a receptor for the Cry11A toxin, and together with its ability to bind an ALP suggest a similar mechanism of toxin action as previously proposed for lepidopteran insects.

Contributed Paper - Wednesday, 08:30 64

Immunohistochemical analyses of *Bacillus thuringensis* toxinbinding proteins in gypsy moth larval gut tissue sections

Algimantas Valaitis - USDA Forest Service, Delaware, Ohio, USA; ***Daniel Krofcheck*** - Ohio Wesleyan University, Delaware, Ohio, USA
Address for correspondence: avalaitis@fs.fed.us

Bacillus thuringiensis (Bt) insecticidal proteins bind to the microvilli on the brush border membranes of gypsy moth gut epithelial cells. This interaction increases epithelial barrier permeability associated with APN shedding and changes the epithelial cell morphology, ultimately resulting in cell swelling and lysis. The presence or absence of specific high-affinity Bt toxin-binding receptors on the microvilli of gut epithelial cells plays a critical role in determining the insecticidal activity of different Bt toxins. Several Bt toxin-binding proteins have been identified as putative

Bt receptors. In the gypsy moth, a 270 kDa glycoconjugate (BTR-270) and one specific membrane-anchored aminopeptidase isozyme (APN-1) have been identified as the two major high-affinity binding proteins for the highly toxic Cry1A Bt toxins. In other insects, cadherins (CADs) and an alkaline phosphatase (ALP) have also been characterized as Bt toxin-binding proteins. In this study, immunohistochemical localization of gypsy moth BTR-270, APN-1, cadherin (LdCAD) and ALP were compared to Bt-toxin binding sites using confocal laser scanning microscopy. Microvilli on the brush border membrane were found to be exclusively decorated with the antibodies directed towards BTR-270 and APN-1 in the midgut and hindgut regions in gypsy moth larval gut sections. The fluorescentlylabeled Cry1A toxin binding sites were found to be co-localized with the gypsy moth toxin-binding receptors BTR-270 and APN-1.

Contributed Paper - Wednesday, 08:45 65

Residues of domain III of Cry1ab toxin from *Bacillus thuringiensis* involved in toxicity and receptor binding

Josue Ocelotl - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Teresa Martinez*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Ivan Arenas*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Sabino Pacheco*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Ricardo Grande*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Alejandra Bravo*** - Instituto de Biotecnologia, UNAM Cuernavaca, Morelos, Mexico; ***Isabel Gómez*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; ***Mario Soberón*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico;
Address for correspondence: isabelg@ibt.unam.mx

Cry toxins are specifically toxic to the larvae of a wide variety of insects. On the basis of the three-dimensional structures of Cry proteins, their active molecules were found to be composed of three domains, i.e., I, II, III. Domains I and II are involved in pore-formation and receptor binding respectively. However, the role of domain III has so far eluded understanding. For some Cry proteins, domain III is important for determining toxicity and insect specificity. Previously we identified antibodies that recognized ?16- ?22 in domain III and we showed that ?16 is involved in the interaction with APN in Manduca sexta. In addition our antibody lowered the toxicity of Cry1Ab toxin to M. sexta. Alanine substitution mutations in the Cry1Ab domain III from amino acid residues 509STLRVN514 (?16 region) and 583VFTLSAHV590(?22), were constructed to study the functional role of domain III in the toxicity and receptor binding with M. Sexta APN-1. All mutants proteins produced stable toxic fragments, and we tested the effect of each mutation on toxicity and binding with M. sexta Aminopeptidase and Alkaline phosphatase receptors .

Contributed Paper - Wednesday, 09:00 66

Cry anti-toxins: A dominant negative phenotype demonstrating that oligomerization is fundamental step in toxin mode of action.

Claudia Rodríguez-Almazán - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, México; ***Liliana Pardo-López*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, México; ***Carlos Muñoz-Garay*** - Instituto de Biotecnologia, UNAM, Montréal, Québec, Canada; ***Luke Masson*** - Biotechnology Research Institute, National Research Council of Canada, Cuernavaca, Morelos, México; ***Mario Soberón*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, México; ***Alejandra Bravo*** - Instituto de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico
Address for correspondence: claudiar@ibt.unam.mx

The Cry toxins produced by *Bacillus thuringiensis* (Bt) are widely used as insecticides. The Cry toxins are produced during sporulation of the bacteria as inclusions crystals. Upon ingestion by the insect larvae and under alkaline conditions in the insect midgut, the crystal inclusion release the monomeric pro-toxins which are activated by midgut host proteases. The toxin monomers bind to receptors, oligomerize and then toxin oligomers bind to a second cry receptor that targets the complex into membrane lipid rafts, where the toxin inserts, forming pores. We hypothesized that mutants of Cry toxins affected in pore formation might be dominant negative. In this work we analyzed eleven mutants affected in different steps of the mechanism of action namely binding to receptors, oligomerization, pore formation and toxicity. The mutants were analyzed for a dominant negative phenotype *in vivo* by bioassays against susceptible *Manduca sexta* larvae and *in vitro* by analyzing pore formation activity in lipid bilayers. We identified several dominant-negative mutants in domain I, and these mutants are able to co-assemble with wild type Cry toxins into hetero-oligomeric structures leading to the total inhibition of membrane insertion and pore-formation activity of wild type toxin. This is the first reported case of a Cry anti-toxin that works as dominant negative inhibitor. The dominant negative mutations clearly demonstrate that oligomerization is a fundamental step in Cry toxin mode of action.

Contributed Paper - Wednesday, 09:15 67

Improvement of Cry toxin insecticidal activity by directed evolution

Volker Heinrichs - Athenix, Research Triangle Park, NC, USA; ***Jayme Williams*** - Athenix, Research Triangle Park, NC, USA
Address for correspondence: vheinrichs@athenixcorp.com

Cry protein endotoxins are expressed in *Bacillus thuringiensis*, and members of this protein family are toxic to Coleoptera, Lepidoptera, and nematodes. Cry toxins form insoluble crystals that are solubilized and in many cases processed by proteolytic cleavage in the insect gut. These toxins are believed to recognize receptors in the insect gut, and act by perforating the gut lining. Transgenic crop plants expressing Cry proteins show improved resistance to insect damage. We used directed evolution to improve the insecticidal activity of *Axmi-R1,* a Cry toxin, against western corn rootworm (*Diabrotica virgifera*) and southern corn rootworm (*Diabrotica undecimpunctata*). *Axmi-R1* wt causes stunted growth, but no insect mortality when fed to western or southern corn rootworm. In a first round of directed evolution, 447 *Axmi-R1* point mutants targeting 34 positions were assayed for activity against western and southern corn rootworm. 8 positions linked to improved insecticidal activity were identified. A second generation library was generated that consists of various permutations of improved point mutants. 573 variants were assayed, and 5 variants were identified that cause mortality of western and southern corn rootworm. Transgenic corn plants expressing improved *Axmi-R1* variants are being generated.

Contributed Paper - Wednesday, 09:30 68

Expression of *Bacillus thuringiensis israelensis* Cry10aa toxin: parasporal body formation, toxicity and synergism with Cyt1AA

Alejandro Hernández-Soto - Centro de Investigación en Biología Celular y Molecular, San José, San José, Costa Rica; ***M. Cristina Del Rincón-Castro*** - División de Ciencias de la Vida, Irapuato, GTO, Mexico; ***Ana M. Espinoza*** - Centro de Investigación en Biología

Celular y Molecular, San José, San José, Costa Rica; ***Jorge E. Ibarra*** - CINVESTAV-Irapuato, Irapuato, GTO, Mexico
Address for correspondence: jibarra@ira.cinvestav.mx

The insecticidal success of Bacillus thuringiensis svar. israelensis against mosquito and blackfly larvae is based on an arsenal of toxins harbored in the parasporal crystal of the bacterium, such as Cry4A, Cry4B, Cry11A and Cyt1A. It is known that a fifth toxin, Cry10Aa, is expressed in very low levels and previous attempts to clone and express it were limited and no parasporal body was formed. In this work, the complete Cry10Aa operon (ORF1, ORF2, and the gap in between) was cloned in the pSTAB vector, under the control of the cyt1A operon and the STAB-SD stabilizer sequence, characteristic of this vector. When the acrystalliferous mutant 4Q7 of B. thuringiensis svar. israelensis was transformed with this construct, ca. 0.9 µm, amorphous parasporal bodies were observed in the mature sporangia under phase contrast and transmission electron microscopy. These parasporal bodies were purified by gradient centrifugation and subjected to SDS-PAGE analyses, showing two major bands of ca. 68 and 56 kDa. These bands were identified by N-terminal sequencing of tryptic fragments using MALDI-TOF-MS analysis. Both bands were the expression products of ORF1 and ORF2, respectively. Bioassays on Aedes aegypti larvae of spore-crystal complex and pure crystals of Cry10Aa estimated LC50s of 2,061 and 239.45 ng/ml, respectively. Also, synergism was detected between Cry10A and Cyt1A, with potentiation rates estimated at 13.3 and 12.63 when mixture of Cyt1A crystals and the Cry10Aa spore-crystal complex; and of Cyt1A and Cry10Aa pure crystals, were tested, respectively.

CONTRIBUTED PAPERS — Wednesday, 08:00 - 10:00
Painted Horse I-II

Fungi I

Moderator: Helen Roy

Contributed Paper - Wednesday, 08:00 — 69

Pathogenicity of the entomopathogenic fungi *Beauveria bassiana* and its effect on the behavior of *Polistes dominulus* wasp

Andrés France - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Loreto Merino*** - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Marcos Gerding*** - INIA Quilamapu, Chillán, Bio Bio, Chile; ***Ricardo Ceballos*** - INIA Quilamapu, Chillán, Bio Bio, Chile
Address for correspondence: afrance@inia.cl

The efficacy of entomopathogenic fungi is not always related to insect mortality but also variations on insect behavior that finally affect its normal biology. Thus, this changes could be more remarkable in social insects, were simple modifications may distort the whole colony. The objective of this research was to evaluate sublethal effect of *Beauveria bassiana,* isolate DW B933, on colonies of *Polistes dominulus* paper wasp. A behavior matrix was build to establish their different activities and the demanding time on healthy wasp colonies and those receiving liquid baits containing $1x10^8$ conidia mL of *B. bassiana.* The Observer® software was used to analyze these activities and their related times. The results showed that wasps fed with *B. bassiana* reduced (p= 0.05) their total time dedicated to normal activities, such as drinking, brooming, nest expansion, larva feeding, flying, walking, and interaction with other individuals. After 11 days these activities decrease by 80% compared with a healthy nest. Consequently, the wasp inactive time increases proportionally, causing an irreversible colony collapse after 10 days due to the lack of feeding, cleaning and nest maintenance.

Contributed Paper - Wednesday, 08:15 — 70

Weakness in the band: nutrient imbalance and immunodeficiency in mass-migrating cannibalistic katydids

Robert Srygley - USDA-Agriculture Research Service, Sidney, MT, United States; ***Patrick Lorch*** - Kent State University, Kent, OH, United States
Address for correspondence: robert.srygley@ars.usda.gov

Mormon crickets (*Anabrus simplex*) form large migratory bands that march over rangeland in the western United States in search of nutrients. Immune defense is particularly relevant to survival in migratory bands, but little is known about the role of nutrition in insect immunocompetence, particularly in the wild. We hypothesized that immune defenses are compromised in Mormon cricket bands due to nutrient limitations. To determine general constituents lacking in their diet, we presented captive members of the band with both protein rich and carbohydrate rich diets. Members of a migratory band in Utah preferred the protein diet, indicating a protein deficiency like that found in Idaho. In contrast, members of the Nevada band preferred the carbohydrate diet, and showed little interest in proteins. These two kinds of nutrient deficiency were associated with different kinds of immunodeficiency. In the protein-deficient band, a protein diet enhanced phenoloxidase (PO) activity, an enzyme involved in wound healing and fighting foreign invasion. PO activity was unaffected by the dietary treatments in the carbohydrate-deficient band. In the carbohydratedeficient band, feeding on carbohydrates enhanced the crickets' ability to encapsulate foreign particles and lyse bacteria, whereas these abilities were unaffected by the dietary treatments in the protein-deficient band. Shortly after feeding on protein or carbohydrates, Mormon crickets exhibited measurable effects on the immune system. The difference in components of the immune system that are enhanced by the contrasting dietary constituents suggests that PO activity requires protein whereas encapsulation and antibacterial activity require carbohydrate fuels. Thus there may not be a common currency for the generalized immunity of insects. In the general framework of ecological nutrition, insects may require a balanced diet to maximize defense against invasion.

Contributed Paper - Wednesday, 08:30 — 71

A *Beauveria bassiana*-based "trap and kill" device to control the major Chagas disease vector in southern South America

Nicolas Pedrini - INIBIOLP (CCT La Plata CONICET-UNLP), La Plata, Buenos Aires, Argentina; ***Juan R. Girotti*** - INIBIOLP (CCT La Plata CONICET-UNLP), La Plata, Buenos Aires, Argentina; ***M. Patricia Juarez*** - INIBIOLP (CCT La Plata CONICETUNLP), La Plata, Buenos Aires, Argentina
Address for correspondence: nicopedrini@yahoo.com

Chagas disease is the most relevant parasitic disease in Latin America, being a major burden that affects mostly poor human populations living in rural areas, with current estimates of ~10 million people infected and ~40 million at risk. The parasite *Trypanosoma cruzi* is mainly transmitted through blood-feeding triatomine bugs. Current strategies to control *Triatoma infestans,* based on residual chemical insecticide application, are threatened by the emergence of pyrethroid-resistance. Among alternative control tools, we investigated the potential of the entomopathogenic fungus *Beauveria bassiana* in the field. We designed a "trap and kill" device based on manipulating *T. infestans* behavior in order to facilitate close contact with a virulent strain of *B. bassiana*. The device consisted of a box containing a CO_2 source, a known blood-sucking insect attractant, combined with a powder formulation of *B. bassiana* conidia and diatomaceous earth. The trap was tested in field assays performed

in 9 houses from two rural villages in the Argentina/Bolivia border infested with pyrethroid-resistant insects. After one intervention, more than 50% of the collected bugs were killed by fungal infection. Based on available *T. infestans* population models, we estimated the impact of the bioinsecticide performance in reducing the risk of acquiring the parasite infection. The potential *T. cruzi* transmission risk index, defined as the maximum number of risky bites a human can receive per night, was estimated to drop from 5.2 to 2.4. According to this model, a second bioinsecticide application is expected to reduce the infection risk to 0.88 bites per human per night, and further decline thereafter. This approach might also prove useful at different settings, e.g. peridomiciliary environments where current tactics and procedures are reported to fail, and rural communities located in remote areas inaccessible to sanitary control teams. These results might help to provide a safe and efficient alternative to overcome bug pyrethroidresilience, and might be useful to control other Chagas disease vectors as well.

Contributed Paper - Wednesday, 08:45 72

Pandora infections in *Formica* ants

David P. Hughes - Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; ***Jørgen Eilenberg*** - Centre for Social Evolution, Department of Agriculture and Ecology, University of Copenhagen, Frederiksberg C, Denmark, Denmark; ***Jacobus J. Boomsma*** - Centre for Social Evolution, Department of Biology, University of Copenhagen, Copenhagen, Denmark, Denmark; ***Annette B. Jensen*** - Centre for Social Evolution, Department of Agriculture and Ecology, University of Copenhagen, Frederiksberg C, Denmark, Denmark
Address for correspondence: jei@life.ku.dk

Ants seem to be the only social insects with specialized pathogens from Entomophthorales associated (the species *Pandora formicae* and *P. myrmecophaga*) making such host-pathogen systems very attractive to study. We have initiated studies (field observations and laboratory experiments) on *Pandora* infections among ants (*Formica rufa* complex and *F. exsecta*) and preliminary data will be presented. Moribund hosts exhibit an extended phenotype, in which ants climb grass or other low vegetation in the proximity to the nests. Mostly the ants turn upside down before fixing themselves to the vegetation by their mandibles. Primary spore discharge takes place from these individuals. Spores from Danish material fit with *P. formicae*. We managed to isolate the fungus *in vitro* in liquid media, and to obtain primary spores. Also, the fungus was successfully transferred to egg-yolk medium. We have compared the genetic identity of the Danish fungus with *Pandora* from infected *Formica* species from The Netherlands and Finland. The Danish and Dutch isolates (both from *F. rufa* complex) were identical (ITS sequences) while the Finnish isolate (from *F. exsecta*) was different.

Contributed Paper - Wednesday, 09:00 73

Overlapping niches of *Beauveria bassiana* in a conifer forest

Helen Roy - Centre for Ecology & Hydrology, Wallingford, Oxfordshire, UK; ***Emma Ormond*** - Anglia Ruskin University, Cambridge, Cambridgeshire, UK; ***Judith Pell*** - Rothamsted Research, Harpenden, Hertfordshire, UK; ***Alison Thomas*** - Anglia Ruskin University, Cambridge, Cambridgeshire, UK
Address for correspondence: hele@ceh.ac.uk

There is limited information on the spatial distribution and diversity of *Beauveria bassiana s.l.* in above-ground locations. The occurrence of *B. bassiana s.l.* in a conifer forest was assessed in October, March and June 2006 through collection of samples from soil, branches and bark and isolation onto selective media. The density of *B. bassiana s.l.* was greater in soil (up to mean (SE) 148.9 (15.5) CFUs per gram) compared to bark (up to mean (SE) 28.8 (14.8) per gram). For branch samples the mean (SE) number of CFUs ranged from 0.0 (0.0) to 42.3 (9.9) per 2 cm low branch and from 0 (0) to 37.3 (16.9) per 2 cm mid branch. In all samples *B. bassiana s.l.* showed a temporal decline from October to June and was completely absent from the conifer branch samples in June. More extreme environmental conditions above-ground compared to below-ground are likely to be the major factor contributing to this decline. Molecular analyses (Inter Simple Sequence Repeat PCR (ISSR-PCR)) indicated that *B. bassiana s.l.* is genetically diverse and there appears to be distinct isolates associated with microhabitats and time; there were separate genetically related clusters of isolates originating from the soil or tree location and separate clustering of October, March and June isolates. Some isolates were ubiquitous in above and below-ground locations. The occurrence of *B. bassiana s.l.* isolates above and below-ground suggests that this fungus occupies various overlapping niches. The heterogeneous nature of the conifer forest provides a model system for studying the population dynamics of this fungus.

Contributed Paper - Wednesday, 09:15 74

Effects of the peptide mycotoxin destruxin a on the renal tubules of *Rhodnius prolixus*

Esau Ruiz-Sanchez - Department of Biology, University of Toronto at Mississauga, Mississauga, Ontario, Canada; ***Ian Orchard*** - University of Toronto at Mississauga, Mississauga, Ontario, Canada; ***Angela Lange*** - University of Toronto at Mississauga, Mississauga, Ontario, Canada
Address for correspondence: e.ruiz.sanchez@utoronto.ca

Entomopathogenic fungi represent an environmentally friendly alternative to control disease vector insects such as Rhodnius prolixus . The study of the interaction between entomopathogenic fungi and their insect hosts has shown that the production of peptide toxins by fungi during the infection process plays critical role in pathogenesis. To contribute to the knowledge on the mechanism of action of these mycotoxins on insect internal organs, we have evaluated the effects of destruxin A, a cyclic peptide produce by Metarhizium anispliae on the fluid secretion rate and transepithelial electrical potential (TEP) of R. prolixus Malpighian (renal) tubules. Destruxin A dramatically inhibited fluid secretion rate on partially (0.05 µmol l^{-1}) and fully (1 µmol l^{-1}) serotonin-stimulated tubules. The calculated IC_{50} for destruxin A on fully serotonin-stimulated tubules was 0.29 µmol l^{-1} . Fluid secretion rate was also strongly inhibited by destruxin A in tubules stimulated with 500 µmol l^{-1} cAMP or 500 µmol l^{-1} dibutyryl cAMP. The use of calcium free saline or addition of $CdCl_2$ to the bathing saline did not interfere with the action of destruxin A, nor did the intracellular calcium antagonist TMB-8. Measurement of TEP on tubules after incubation for 10 min in saline containing 5 µmol l^{-1} destruxin showed that the second phase of the typical triphasic response to serotonin is disrupted. Taken together, these results show that destruxin A inhibits fluid secretion rate by the Malpighian tubules of R. prolixus by a mechanism downstream of the effects of serotonin or cAMP. In addition, extracellular and intracellular Ca^{2+} do not participate in the inhibitory effect of destruxin.

Contributed Paper - Wednesday, 09:30 75-STU

Hydrophobins of the entomopathogenic fungus *Beauveria bassiana*

Shizhu Zhang - University of Florida, Gainesville, FL, USA; ***Nemat Keyhani*** - University of Florida, Gainesville, FL, USA

Address for correspondence: shizhuz@126.com

Hydrophobins are small amphipathic proteins found that function in diverse physiological roles in filamentous fungi, ranging from adhesion to development to virulence. The genes for two hydrophobins, *hyd1* and *hyd2*, have been previously isolated from *B. bassiana.* Molecular and biochemical analysis of these genes and their protein products revealed that *hyd1* was highly expressed during various developmental stages of the fungus, whereas *hyd2* was implicated as constituting the major component of the *B. bassiana* conidial rodlet layer. In order to further probe the functions of these proteins, targeted gene-knockouts of *hyd1* and *hyd2* were constructed by homologous recombination and deletion of a portion of the open reading frames of each gene via insertion of the phosphinothricin resistance marker (bar). Mutant insertion sites were verified by PCR and Southern blots, and loss of corresponding transcripts confirmed by RT-PCR. The effects of *hyd1* and *hyd2* on surface hydrophobicity, distribution of surface carbohydrates, fungal development and virulence was determined.

Contributed Paper - Wednesday, 09:45 76

A selective medium for isolating entomopathogenic fungi *Metarhizium* and *Beauveria* from Western United States soil

Éverton K. K. Fernandes - Utah State University, Logan, UT, USA; ***Chad A. Keyser*** - Utah State University, Logan, UT, USA; ***Drauzio E. N. Rangel*** - Utah State University, Logan, UT, USA; ***R. Nelson Foster*** - USDA/APHIS/PPQ/CPHST Lab, Phoenix, AZ, USA; ***Donald W. Roberts*** - Utah State University, Logan, UT, USA
Address for correspondence: dwroberts@biology.usu.edu

This study focuses on a search for new fungal isolates with greater potential than the currently available commercial isolates to control Mormon crickets (MC), *Anabrus simplex*, and other orthopteran pests. A primary objective of this survey is to isolate an Orthoptera-host-specific fungus, *M. anisopliae* var. *acridum* (*Ma-ac*), from US soil. Conidia from fungus-killed MC/Grasshopper (GH) cadavers are routinely deposited on or in nearby soil. Our survey, therefore, has centered on isolating fungi from soil collected in MC/GH habitats. Currently, the most used selective medium for *Metarhizium* and *Beauveria* isolation is based on nutritive mycological agar supplemented with the fungicide n-dodecylguanidine acetate (Dodine) plus the bactericide gentamicin. Although this medium efficiently selects spores of *Metarhizium* and *Beauveria* when mixtured with non-entomopathogenic fungi, this medium proved ineffective in isolating some important species of entomopathogenic fungi from soil, particularly *Ma-ac*. Accordingly, we developed a selective medium for isolation of *Metarhizium* and *Beauveria* from soil. The medium, designated CTC medium, consists of potato dextrose agar plus yeast extract (PDAY) supplemented with chloramphenicol, thiabendazole and cycloheximide. This medium afforded efficient isolation of *Ma-ac* from soil samples inoculated in the laboratory with fresh conidia. Since 2008, we have processed 7,040 soil samples collected from most states of the western United States. Most of the soil samples were collected by the MC/GH Population Survey Teams of each state through a cooperative agreement with USDA/APHIS. Presently, using PDAY-CTC medium, we have isolated 219 new *Metarhizium* and 480 new *Beauveria* isolates from western United States (*Metarhizium*: 5 Arizona, 11 Colorado, 2 Montana, 144 Nebraska, 18 North Dakota, 3 Oregon, 12 South Dakota, 5 Texas, 5 Utah, 12 Washington, 2 Wyoming; *Beauveria*: 45 Arizona, 5 California, 52 Colorado, 10 Montana, 78 Nebraska, 3 North Dakota, 10 Oklahoma, 10 Oregon, 25 South Dakota, 42 Texas, 183 Utah, 13 Washington, 4 Wyoming). The identifications were based on morphology, and the isolates are stored at low temperature. Our previous studies have shown that *Ma-ac* conidia have high tolerance to 8h at 45ºC. In contrast, other *Metarhizium* varieties do not survive this level of heat treatment. Accordingly, conidial suspensions of several the new *Metarhizium* isolates have been exposed to 45ºC for 8h for putative identification purposes. The new isolates have been or will be identified using DNA amplification and sequencing techniques. Additionally, the isolates' virulence to MC will be determined in laboratorial assays, and they will be tested for high tolerance to environmental conditions, e.g. heat and UV radiation, routinely encountered in MC habitats.

Symposium (Bacterial Division) Wednesday, 10:30 - 12:10
Kokopelli Ballroom II

Bt the Bacterium, Ecology and Infection

Organizer/Moderator: Christina Nielson-LeRoux

Symposium - Wednesday, 10:30 77

Bacillus thuringiensis: in vivo development, plasmid conjugation and expression of virulence and adaptation factors

Christina Nielsen-LeRoux - INRA, Guyancourt, Yvelines, France; ***Nadine Daou*** - INRA, Guyancourt, Yvelines, France; ***Fuping Song*** - CASS, Bejing, China; ***Clelton Santos*** - Dept. Biologia Geral, Londrina, PR, Brazil ; ***Gyslaine Vilas-Boas*** - Dept. Biologia Geral, Londrina, PR, Brazil ; ***Christophe Buisson*** - INRA; ***Jie Zhang*** - Institute of Plant Protection, CAAS, Beijing, China, ***Olivia Arantes*** - Universidad Estadual de Londrina, Brazil, ***Didier Lereclus*** - INRA
Address for correspondence: Christina.nielsen@jouy.inra.fr

The capacity of *B. thuringiensis (Bt)* and the related *B. cereus (Bc)* to infect and multiply during infection of insect larvae are dependent on various factors more or less reliant to the presence of the Cry toxinencoded plasmids. The presentation will cover results from various approaches aiming to identify factors enableling the bacteria to infect, develop, persist and fullfill the lifecycle inside the insect. We also investigated on conjugation capacities of Bt and Bc strains in relation to the bacterial cell cycle in order to understand whether this can explain eventual ecological niches of Bt and Bc. To study the role of chromosomal genes of Bt and Bc we used the Bt407 crystal minus strain and the sequenced Bc ATCC14579 strain. Virulence of these strains is mainly due to factors from the PlcR regulon (Salamitou et al. Microbiology, 2000). Gene expression was analysed in *Galleria mellonella* and conjugation experiments were run with *Bombyx mori.* An IVET (in vivo expression technology) promoter–trap system, (Fedhila et al.Mol.Microbiol. 2006), and transcriptional gfp-fusions based on selected genes from IVET and the PlcR regulon were used to analyse gene expression. From IVET, we focused on a surface protein expressed in the hemocoel, named IlsA, which is essential for iron acquisition and virulence. The second IVET-gene is a part of a new sugar phosphate sensing system (SPS) found to be exclusively expressed in the midgut. The gfp fusions showed that genes from the PlcR regulon are modulated during infection compared to in vitro conditions. The conjugation efficiency in bi- and triparental systems (± Cry toxins) run in vitro and in vivo, showed that Bt germinated and multiplied more efficiently than Bc strains and that the larval environment enhanced plasmid transfer. Thus, our results show that some Bt-specific factors are important for the adaptation to the invertebrate environment.

Symposium - Wednesday, 11:00 78

Bacillus thuringiensis toxins as cooperative public goods--can

social evolution theory explain the dynamics of *Bt* virulence

Ben Raymond - University of Oxford, Oxford, Oxfordshire, United Kingdom
Address for correspondence: benjamin.raymond@zoo.ox.ac.uk

Bt toxin production is highly metabolically costly and the means by which this trait is maintained in *Bacillus* populations is still controversial. If we treat Bt toxins are cooperative traits some of this controversy may be resolved. Bt toxins behave according to the definition of a cooperative "public good". Within hosts avirulent crystal null strains (or "cheats") can exploit the toxins produced by virulent strains, and the growth rate of cheats within hosts is higher than that of virulent strains. However, toxin production is essential for pathogenicity and cheats are not expected to be able to persist in the absence of toxin producers. Kin selection and competition between patches with varying proportions of toxin-production can however, explain the maintenance of this trait. In a field experiment I competed near-isogenic virulent and avirulent cheats at varying densities and frequencies. Evidence for a kin-selection process was found in strong negative frequency dependence, in other words cheats rapidly invaded populations with high frequencies of toxin-producers, and toxin-producers rapidly invaded populations with high frequencies of cheats. Evidence of competition between cheats and toxin-producers was also found in the spatial distribution of these phenotypes across the experiment. Not only can Bt be used as a tool to test to evolutionary theory but the evolutionary tension between cheats and toxin-producers can explain the phylogenetic distribution of toxin production as well as the rarity of Bt epizootics in the field.

Symposium - Wednesday, 11:30 79

The role of mid-gut flora in the pathogenicicity of *Bacillus thuringiensis* to susceptible and resistant Lepidoptera

Paul R. Johnston - Department of Biochemistry, School of Life Sciences, Falmer, Brighton, United Kingdom; ***Ben Raymond*** - Mathematical Ecology Research Group, Dept of Zoology, South Parks Road, Oxford, United Kingdom; ***Vidisha Krishnan*** - Department of Biochemistry, School of Life Sciences, Falmer, Brighton, United Kingdom; ***Neil Crickmore*** - Department of Biochemistry, School of Life Sciences, Falmer, Brighton, United Kingdom; ***Denis J. Wright*** - Division of Biology, Faculty of Natural Science, Silwood Park Campus, Ascot, United Kingdom; ***Richard J. Ellis*** - Molecular Pathogenesis and Genetics, Veterinary Laboratories Agency, UK; ***Michael B. Bonsall*** - Mathematical Ecology Research Group, Dept of Zoology, University of Oxford, UK,
Address for correspondence: n.crickmore@sussex.ac.uk

In 2006 Broderick et al (PNAS 103:15196-15199) published a thoughtprovoking paper in which they claimed that the toxicity of *Bacillus thuringiensis* towards the Gypsy Moth was largely or wholly due to the effect of native bacteria within the gut of the insect larvae. We were interested in seeing whether differences in the gut flora could account for differences in susceptibility of different populations of Diamondback Moth to Bt or its toxins. No correlations could be found although we did replicate the findings of the above paper in that populations of Diamondback Moth whose gut bacteria had been removed through treatment with antibiotics were less susceptible to spore/crystal formulations of Bt. We then extended these studies to see whether the same results could be obtained using antibiotic-resistant strains of Bt and/or populations of insect whose gut bacteria had been removed through sterile techniques rather than through the use of antibiotics. We will present the results of these analyses in this talk.

Symposium - Wednesday, 12:00 80

***Bacillus thuringiensis*, resident gut microbiota, and innate immunity in lepidopteran insects**

Jo Handelsman - UW-Madison, Dept. of Bacteriology, Madison, WI, USA; ***Kenneth Raffa*** - UW-Madison, Dept. of Entomology, Madison, WI, USA; ***Nichole Broderick*** - Global Health Institute,
Address for correspondence: joh@bact.wisc.edu

The goal of this work is to determine mechanisms by which *Bacillus thuringiensis* (Bt) toxin kills lepidopteran larvae following initial disruption of the gut. Until recently, two leading hypotheses were that death is caused either by host starvation or bacteremia associated with Bt cells in the insect's hemolymph. Since starvation in some species is much slower than death by Bt toxin and the toxin is lethal in the absence of Bt cells, we explored other mechanisms, focusing on whether gut bacteria might reduce or contribute to susceptibility. We found that Bt is unable to kill five species of Lepidoptera in the absence of enteric bacteria, and restoring these bacteria in the gut also restores Bt lethality for four of them. A sixth species, pink bollworm, showed higher mortality when given antibiotics, and also has a substantially different gut community. Exploring gypsy moth in more detail showed that this relationship held regardless of whether Bt was administered in formulations containing or not containing cells, or through engineered E. coli, that Bt cells grew in dead or moribund but not live larvae, and that there was no evidence for direct effects of antibiotics on the insect or Bt. Recent work by other researchers showed that ingestion of Bt increases oxidative stress levels in lepidopteran midguts. Based on this and our own data, we speculated that perturbation of the insect's innate immune response by Bt in combination with the normal gut microbiota may contribute to death. We found that gypsy moths fed Bt experienced depletion and abnormalities of hemocytes prior to death. Moreover, pharmacologic agents that modulate innate immune responses altered the time required for larval mortality by Bt. We will discuss the implications of these results for models of toxicity of Bt toxin.

CONTRIBUTED PAPERS Wednesday, 10:30 - 12:30
Painted Horse I-II

Nematodes II

Moderator: Bishwo Adhikari

Contributed Paper - Wednesday, 10:30 81-STU

***Thripinema fuscum* parasitism reduces the vector competency of *Frankliniella fusca* to transmit tomato spotted wilt virus**

Kelly Sims - Department of Entomology and Nematology, Gainesville, Florida, USA; ***Joseph Funderburk*** - North Florida Research and Education Center, Quincy, Florida, USA; ***Stuart Reitz*** - Center for Medical, Agricultural and Veterinary Entomology, Tallahassee, Florida, USA; ***Drion Boucias*** - Department of Entomology and Nematology, Gainesville, Florida, USA
Address for correspondence: simsk@ufl.edu

The tobacco thrips, *Frankliniella fusca* (Thysanoptera: Thripidae), is a polyphagous insect pest that vectors Tomato spotted wilt virus (TSWV, Bunyaviridae: Tospovirus) to numerous vegetable and ornamental crops. The discovery of the insect parasitic nematode *Thripinema fuscum* (Tylenchida: Allantonematidae) parasitizing populations of *F. fusca* implicated it as a potential biological control agent of viruliferous thrips. Significantly, in nature *T. fuscum* causes near-extinction of local *F. fusca* populations by sterilizing female thrips. It has recently been demonstrated that *Thripinema* impacts the vector competence of *F. fusca* to transmit TSWV by inducing a host behavioral response that reduces

feeding rates. Additionally, a qRT-PCR assay, directed at detecting the conserved sequence of n-protein, was developed to quantify and compare TSWV viral titers between viruliferous non-parasitized and parasitized *F. fusca* females. Results demonstrated that parasitism by *Thripinema* also induces an altered physiological response in host thrips that impacts their vector competence by reducing viral pathogen titers. This is the first known example of a parasite reducing the vectoring capability of an insect to transmit a plant pathogen. The potential mechanisms regulating the ability of these parasites to interfere with viral competence of insect vectors will be discussed.

Contributed Paper - Wednesday, 10:45 82-STU

Aggregation behavior in adults of *Steinernema carpocapsae*

Yolanda Reyes - Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, Mexico; ***Ali Asaff*** - Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, Mexico; ***Mayra De la Torre*** - Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, Mexico
Address for correspondence: yorevi@estudiantes.ciad.mx

In *in-vitro* monoxenic solid culture of *Steinernema carpocapsae*, the mature females always form groups of over 2 worms and only males are arrested in these groups. Males wander on the plate and only females aggregate into groups when separated from the culture where they developed since infective juveniles. This behavior known as aggregation was not observed in any other stage of *S. carpocapsae* development. We found that male aggregation behavior is only observed when a factor originating of females is present. In this study, factors analyzed were: live females, dead females and males, human hair, females washed with organic solvents and macerated females or males. These factors can be group as physical (tactile communication) or chemical (pheromone communication), when both factors (live females) are present the maximum aggregation was observed. In order to characterize the pheromone mediated aggregation in adults of *S. carpocapsae*, we obtained an active fraction using chromatographic fractionation of excretory and secretory products included in the macerated of females. These substances can be classified as semiochemicals by the effect between adults of the same species of nematode.

Contributed Paper - Wednesday, 11:00 83-STU

Rate of lateral dispersal of the entomopathogenic nematode *Heterorhabditis bacteriophora* from infected host cadavers in soil

Harit K. Bal - Department of Entomology, OARDC, The Ohio State University, Wooster, OH, USA; ***Robin R.A.J. Taylor*** - Department of Entomology, OARDC, The Ohio State University, Wooster, OH, USA; ***Parwinder S. Grewal*** - Department of Entomology, OARDC, The Ohio State University, Wooster, OH, USA
Address for correspondence: bal.9@osu.edu

The rate of lateral dispersal of *Heterorhabditis bacteriophora* GPS11 strain from infected host cadavers was quantified in 5 cm deep autoclaved soil with 24% moisture content placed in wooden trays at room temperature (21°C). Each of the three experiments had different sized trays: 22.86 cm x 22.86 cm, 61 cm x 61 cm and 122 cm x 122 cm for experiments 1, 2 and 3, respectively. A single 10-day old cadaver of final instar *Galleria mellonella* infected with 420 infective juveniles of *H. bacteriophora* was placed in the center of each tray. Soil core samples (2 cm dia and 5 cm deep) were collected in plastic cups at different intervals from 6 to 240 hours and at different distances from 3.81 to 61 cm from the cadaver and an uninfected *G. mellonella* larva was placed in each cup to examine nematode infection three days later. Each experiment was replicated five times and all three experiments were repeated. The spatio-temporal data were analyzed by a two-dimensional modified Fick Diffusion Model with least squares method. Average movement of infective juveniles was 6 cm/day. Number of infective juveniles moving a given distance declined with increasing distance from the cadaver with 40% traveling >15 cm and 2.5% traveling >60 cm in 240 hours. This study revealed remarkable innate ability of *H. bacteriophora* to move in soil in the absence of a host.

Contributed Paper - Wednesday, 11:15 84

Tritrophic trade-offs affecting growth and resistance to pathogens for a polyphagous herbivore.

Aaron Gassmann - Iowa State University, Ames, IA, USA; ***Patricia Stock*** - University of Arizona, Tucson, AZ, USA; ***Bruce Tabashnik*** - University of Arizona, Tucson, AZ, USA; ***Michael Singer*** - Wesleyan University, Middletown, CT, USA
Address for correspondence: aaronjg@iastate.edu

Plant-mediated trade-offs between growth of herbivorous insects and their resistance to parasitoids have received recent study, but less is known about how host plants affect resistance of herbivores to pathogens. We compared growth and resistance to entomopathogenic nematodes for woolly bear caterpillars *Grammia incorrupta* fed lettuce *Lactuca sativa* versus threadleaf groundsel *Senecio longilobus*. Both plants are members of the Asteraceae, but only *Senecio* contains pyrrolizidine alkaloids. Caterpillars grew faster when fed *Lactuca* versus *Senecio*, yet in one of four cases studied, caterpillar resistance to nematodes was higher when fed *Senecio* versus *Lactuca*. Caterpillar resistance to nematodes did not differ between host plants in the other cases. Nematode reproduction was higher in cadavers of *Grammia* that had been fed *Lactuca* instead of *Senecio*. Our results illustrate how host plants may impose trade-offs affecting growth of herbivorous insects and survival of herbivores in the presence of pathogens. Such trade-offs may act to structure plant-insect interactions by selecting for wider diet breadth of herbivores.

Contributed Paper - Wednesday, 11:30 85

Soil microarthropod response to the application of *Steinernema carpocapsae*–killed insects in maize and refuge habitats.

Randa Jabbour - Washington State University, Pullman, WA, USA; ***Mary Barbercheck*** - Pennsylvania State University, University Park, PA, USA
Address for correspondence: rjabbour@wsu.edu

Insects killed by entomopathogenic nematodes and their symbiotic bacteria represent a resource with which soil arthropods can interact. These interactions can be positive for the nematode (e.g., soil arthropods serve as parasitic or phoretic hosts) or negative (e.g., nematodes serve as prey items). Plant diversity and soil disturbance, which alter the physical and biotic environment, may influence these interactions. We investigated the effects of the presence of maize and refuge habitats on microarthropod abundance and community composition in soil surrounding wax moth larvae, *Galleria mellonella*, infected with *Steinernema carpocapsae* (Sc). In the first year of the experiment (2005), we compared microarthropod communities responding to burial of Sc-killed insects and to no soil disturbance. In 2006, we added two treatments to control for addition of a resource and disturbance: burial of freezer-killed insects and sham burial. Soil samples (including *G. mellonella*) were collected 2 and 20 days (2005) or 2 and 12 days (2006) after application. Samples were placed in Tullgren funnels to extract microarthropods for quantification and identification. In 2005, arthropod abundance and community composition was similar between maize and refuge sites. In 2006, we

detected more arthropods, particularly ascid and *Eupodes* mites, in the maize compared with the refuge. In both years, we observed the greatest difference in community composition between treatments on the final sampling date. Community composition differed between treatments providing resources (Sc-killed and freezer-killed insects) and those without (sham burial and no disturbance). Soil surrounding EPN-killed and freezer-killed insects contained more dipteran larvae, acarid mites, staphylinid beetle larvae, onychiurid and entomobryid collembolans, and mesostigmatid immature and male mites than soil at sham burial and no disturbance sites.

Contributed Paper - Wednesday, 11:45 86

Entomopathogenic nematodes in Tanzania.

Solveig Haukeland - Bioforsk, Norwegian Institute for Agricultural and Environmental Research, As, Akershus, Norway; ***Suma Mwaitulo*** - Sokoine University of Agriculture, Morogoro, Morogoro, Tanzania; ***Anne Laudisoit*** - CERVA-CODA, Uccle, Brussels, Belgium; ***May-Guri Saethre*** - Bioforsk, Norwegian Institute for Agricultural and Environmental Research, As, Akershus, Norway; ***Amon Maerere*** - Sokoine University of Agriculture, Morogoro, Morogoro, Tanzania; ***Khuong Nguyen*** - University of Florida, Entomology and Nematology Dept, Florida, USA
Address for correspondence: solveig.haukeland@bioforsk.no

The occurrence of entomopathogenic nematodes in Tanzania is reported for the first time based on a survey carried out in the Southern and Eastern zones of the country. The survey was carried out in banana plantations where the main intention was to obtain some knowledge on the occurrence of indigenous entomopathogenic nematodes for further studies on their effect against the banana weevil (*Cosmopolites sordidus*). Entomopathogenic nematodes were recovered from 4 samples (4.4%) all from the same region (Eastern Coastal zone). Nine isolates (the progeny from a single *G. mellonella* larva was considered one isolate) were isolated from the four soil samples. The majority of isolates were in the genus *Steinernema,* but *Heterorhabditis* spp. was also present. Among the *Steinernema* isolates at least one new species has been identified so far which appears to be closely related to *Steinernema karii,* associated with as yet unidentified *Xenorhabdus* species closely related to *X. szentirmaii.* Out of six basic soil textural classes, nematodes were recovered from loamy sand and sandy loam soils only. No nematodes were recovered from heavy soils such as sand clay loam and sand clay. In laboratory bioassays it was shown that all the isolates were able to infest banana weevil larvae reaching 100% mortality at the highest dose. Bioassays against banana weevil adults were not successful, none of the isolates caused significant mortality.

Contributed Paper - Wednesday, 12:00 87-STU

Isolation and characterization of a North American species of *Phasmarhabditis.*

Nathaniel Keplinger - Department of Biology, Brigham Young University, Provo, UT, USA; ***Byron Adams*** - Department of Biology, Brigham Young University, Provo, UT, USA
Address for correspondence: natekeplinger@gmail.com

The genus *Phasmarhabditis* is a unique nematode that parasitizes various mollusc species. In Europe it has been used as a biological control agent against slugs and snails since 1994, but implementation in North America has not been achieved because indigenous strains or species that effectively kill molluscs have not been recovered. Recently we recovered an isolate of *Phasmarhabditis* sp. in Utah. The nematodes were identified based on morphology and DNA sequences of the D2/D3 expansion region of the large ribosomal subunit. The sequences obtained are identical to those of a strain of *Phasmarhabditis* (EM434) from New York. The Utah strain was originally isolated via soil baiting methods using *Galleria mellonella*. Virulence to snails and slugs was confirmed in the laboratory by exposure to a liquid suspension of the nematodes applied to filter paper in 60mm Petri dishes. The slugs and snails were killed within 24-48 hours. After 4-10 days slug and snail cadavers were placed on White traps and given up to a week for juvenile nematodes to emerge. All of the cadavers yielded living, viable nematodes. To ensure that *Phasmarhabditis*, and not some other contaminating species was responsible for mortality, several individual nematodes were sampled from the established culture for DNA sequence analysis. The isolate has been maintained for over a year, cycled alternately through insect and mollusc hosts. We are currently investigating aspects of virulence through titration experiments and screens for symbiotic and/or phoretic bacteria.

CONTRIBUTED PAPERS Wednesday, 10:30 - 12:30
White Pine I-II

Microbial Control I

Moderator: Sunday Ekesi

Contributed Paper - Wednesday, 10:30 88-STU

Heat-induced post-stress growth delay: a biological trait of many *Metarhizium* isolates that may reduce field efficacy.

Chad A. Keyser - Department of Biology, Utah State University, Logan, Utah, USA; ***Éverton K. K. Fernandes*** - Department of Biology, Utah State University, Logan, Utah, USA; ***Stefan T. Jaronski*** - USDA ARS NPARL, Sidney, MT, USA; ***Donald W. Roberts*** - Department of Biology, Utah State University, Logan, Utah, USA
Address for correspondence: chadalton@biology.usu.edu

Traditionally, selection of fungal isolates for use against pest insects is based exclusively on high virulence to target insects. Recent research, however, mandates a broadening of the selection traits to include each isolate's susceptibility to environmental stress. Many pest insects occur in temperate regions that commonly have fluctuating temperatures which can range from 0º to over 40ºC within a single day. It is generally assumed that a fungal isolate is unlikely to infect insects at temperatures above its growth threshold; but, as long as the fungus survives, disease development will proceed during cooler periods of the day. A detailed study of ten *Metarhizium anisopliae* isolates, however, made clear that for several isolates mere survival after exposure to high temperatures may not be sufficient to allow infection in field situations where daily temperatures repeatedly exceeded 40?C. This is due to a phenomenon we label "heat-induced post-stress growth delay" (PSGD). We detected PSGD in several *M. anisopliae* isolates, including some that excelled in other important selection criteria. Each isolate was evaluated for virulence toward Mormon crickets (*Anabrus simplex*); conidial production capability; conidial germination; rate of vegetative growth; tolerance to wet-heat (45ºC); and susceptibility to UV-B radiation. A few isolates excelled in many of these categories, and were tentatively selected as candidates for field use. Heat-induced PSGD was evaluated by exposing three-day-old colonies to 40ºC for 4 or 8 hours followed by 20 or16 hours at 28ºC, respectively, for three days, followed by constant 28ºC. Growth rates (mm/d) during heat treatments were compared to control plates (constant 28ºC) and to plates with intermittent growth stoppage by cold treatment (4 or 8h at 5ºC per day). While all isolates survived 3 days of cycled heat treatment and resumed normal growth afterwards; some

isolates, however, were considerably more affected by heat-cycling than others. Two isolates, DWR 346 and DWR 356 (which demonstrated promise in other traits, including virulence and heat tolerance), had their growth rates reduced by 18.6 and 14.2% after 4h/d, and 87.2 and 79.9%, with 8h/d treatment, respectively. Conversely, isolates that had relatively low growth rates at constant 28ºC (i.e., ARSEF 324, DWR 312, and DWR 338) resumed normal growth rates between heat pulses. Fluctuating temperatures are likely to be a factor in most pest-insect habitats; therefore, in addition to evaluating spore survival, PSGD should be a primary consideration for field-appropriate isolate selection.

Contributed Paper - Wednesday, 10:45 89

withdrawn

Contributed Paper - Wednesday, 10:45 90-STU

Can *Steinernema sp.* be lured by an environmental bacterium? A case study with new isolates from Tanzania.

Anne Laudisoit - Veterinary and Agrochemical Research Centre, Brussels, NA, Belgium; ***Solveig Haukeland*** - Norwegian Institute for Agricultural and Environmental Research, As, NA, Norway; ***Pierre Wattiau*** - Veterinary and Agrochemical Research Centre, Brussels, NA, Belgium
Address for correspondence: anne.laudisoit@var.fgov.be

While most entomopathogenic nematodes (EPNs)of the *Steinernema* genus are associated with a relatively specific strain of symbiotic bacteria, some do harbour a common, even ubiquitous, *Xenorhabdus* species. The conditions that favour the strength of the host-symbiont association in those EPNs are not fully understood but the natural loss of *Xenorhabdus* has been observed in some isolates at cold temperatures. Following the identification of new isolates of tropical *Steinernema sp.,* a new *Xenorhabdus* strain was isolated. The strain had an orange pigmentation on TSA and hydrolized aesculine on a special selective medium. The 16S and 18S sequence analysis of,respectively, the bacterium and the nematode lead to the description of a new 'host-entropathogenic bacterium' complex. Classically, the EPNs entire adult life stage only occurs inside the infected target insects. During this study, a new *Steinernema* isolate was cultivated in *Galleria mellonella* larvae but, upon emergence of the infective juveniles, some adult females were observed making attempts to feed outside the decaying larval bodies. Since the *Xenorhabdus* genus is close to the *Yersinia* genus, we tested *in vitro* the possibility to lure feeding adults of one particular *Steinernema* isolate with the human pathogenic *Yersinia pseudotuberculosis* . Indeed, *Y. pseudotuberculosis* is an opportunistic environmental bacterium with nearly worldwide distribution that forms a biofilm around the head of *Caenorhabditis elegans* in vitro. However, since *C.elegans* is not an entomopathogenic species, it might be less resistant to competitive bacteria present in the environment than entomopathogenic species that have to fight hosts defenses and bacterial flora to complete their lifecycle. The results are discussed in an integrated context assessing the risk to use EPNs as biocontrol agents and the public health risk associated with such use.

Contributed Paper - Wednesday, 11:00 91

Identification and blend effects of repellent volatiles of entomopathogenic fungi towards the termite *Macrotermes michaelseni.*

David M. Mwangi - International Centre of Insect Physiology and Ecology (icipe), Nairobi, Nairobi, Kenya; ***Nguya Kalemba Maniania*** - International Centre of Insect Physiology and Ecology (icipe), Nairobi, Nairobi, Kenya; ***Ahmed Hassanali*** - International Centre of Insect Physiology and Ecology (icipe), Nairobi, Nairobi, Kenya; ***Peter A. Njagi*** - International Centre of Insect Physiology and Ecology (icipe), Nairobi, Nairobi, Kenya; ***Linus M. Gitonga*** - Jomo Kenyatta University of Agriculture and Technology, Nairobi, Nairobi, Kenya
Address for correspondence: nmaniania@icipe.org

Volatiles from fungal isolates are believed to be implicated in avoidance behavior towards termite, *Macrotermes michaelseni.* However, the role of the individual odor constituents is still not understood. This hypothesis was tested in the laboratory using synthetic constituents of the fungal isolates against the insect in subtraction assays of the constituents' blends. Volatile constituents of the most and the least repellent isolates of *Metarhizium anisopliae* and *Beauveria bassiana* were identified by GC-MS and GC co-injection with authentic standards. The repellency dose responses of selected blends of 10 major components of the corresponding isolates towards *M. michaelseni* were compared with those of different components missing. The components in blends were assayed in amounts and proportions present in nature at 50% repellency dose levels using Y-olfactometer. Six constituents of volatile blends from isolates of the most and the least repellent fungal isolates were found to be largely responsible for observed repellency. Moreover, results of different blends of six major components of the corresponding isolates, which showed significant drop in repellency after individual exclusion in blends, gave insight that the repellency action were due to combined effects of the different constituents. The significance of the results and their implication in behavior, control and management of termites are highlighted.

Contributed Paper - Wednesday, 11:15 92-STU

Influences of local and long-distance dispersal on the evolution of *Bt* resistance in cabbage looper populations.

Michelle Franklin - Dept. of Zoology, University of British Columbia, Vancouver, BC, Canada; ***Judith Myers*** - Dept. of Zoology, University of British Columbia, Vancouver, BC, Canada; ***Carol Ritland*** - Dept. of Forest Sciences, University of British Columbia, Vancouver, BC, Canada
Address for correspondence: franklin@zoology.ubc.ca

Continued use of *Bacillus thuringiensis* (Bt) products in vegetable greenhouses in British Columbia (BC), Canada is threatened by the rapid evolution of resistance in *Trichoplusia ni* (cabbage looper). The spatial and temporal patterns of Bt resistance in greenhouse and field populations strongly suggest that resistant moths disperse from greenhouses treated extensively with Bt to 'unselected' neighbouring greenhouse populations early in the growing season. To quantify dispersal patterns, we performed genetic analyses using amplified fragment length polymorphism (AFLP) techniques and mitochondrial sequence variation. This confirms significant movement of moths between greenhouses located within 20 km of one another. Most field populations in BC are initiated each spring by long-range migrants from California. Populations surveyed along the migration pathway remain susceptible to Bt and therefore have the potential to dilute resistance in BC greenhouse populations. Molecular analysis suggests that significant genetic homogeneity of populations occurs over the migration path. However, field and greenhouse populations in areas of BC, with a high density of greenhouses, some of which support overwintering populations, remain genetically distinct from long-range migrants. In BC therefore field populations of *T. ni* are a mosaic of those originating from migrants from the south and those influenced by migration from greenhouses.

CONTRIBUTED PAPERS Wednesday, 10:30 - 12:30
Kokopelli Ballroom III

Virus II

Moderators: Rollie Clem, Jim Slavicek

Contributed Paper - Wednesday, 10:30 93

Polydnaviruses as organelles– An alternative paradigm.

Brian Federici - Department of Entomology, Riverside, California, United States; ***Yves Bigot*** - Laboratoire d'Estude des Parasites Genetiques, Tours, France
Address for correspondence: brian.federici@ucr.edu

For almost three decades, particles produced by female parasitoid wasps to circumvent the innate immune system of their insect hosts have been considered to be those of unusual types of viruses known as polydnaviruses (family *Polydnaviridae*). Two genera are recognized, *Bracovirus* and *Ichnovirus*, each consisting of numerous species. When the family was originally proposed, structural and biochemical evidence suggested that the particles were those of viruses. However, recent studies, especially of particle DNA, referred to as the viral genome, as well as studies of wasp genomic DNA and particle function, show that although the particles originated evolutionarily from viruses, their biology is much more consistent with that of organelles that evolved by symbiogenesis. By definition, symbiogenesis is an evolutionary extension of a symbiotic relationship during which two genomes fuse, partially or fully, to produce a new organism with traits different from either of the original partners. Thus, the original partners no longer exist as distinct species. Known examples of symbiogenesis include the evolution of mitochondria and chloroplasts, eukaryotic organelles that evolved from bacteria. Genomic data published over the past few years, as well as studies of particle function provide strong support that polydnaviruses are not viruses but rather a unique type of immunosuppressive organelle. More specifically, the particle DNA is not a viral genome, as it consists almost exclusively of wasp genes and non-coding wasp DNA. Instead, consistent with symbiogenesis, the genes coding for particle structural proteins and enzymes, though of viral origin, are now part of wasp genomes. Functionally, while the particles can enter cells, they do not replicate or produce progeny, key properties of all viruses. Whereas it has been suggested that the definition of a virus should be expanded to accommodate polydnaviruses, a more rationale change would be to adopt an organelle paradigm for these interesting particles.

Contributed Paper - Wednesday, 10:45 94-STU

Proteomic analysis of Chilo iridescent virus particles.

Ikbal Agah Ince - Wageningen University and Research Centrum, Wageningen, Gelderland, the Netherlands; ***Sjef A. Boeren*** - Wageningen University and Research Centrum, Wageningen, Gelderland, The Netherlands; ***Monique M. van Oers*** - Wageningen University and Research Centrum, Wageningen, Gelderland, the Netherlands; ***Jacques J.M. Vervoort*** - Wageningen University and Research Centrum, Wageningen, Gelderland, the Netherlands; ***Just M. Vlak*** - Wageningen University and Research Centrum, Wageningen, Gelderland, the Netherlands
Address for correspondence: agah.ince@wur.nl

Insect iridoviruses infect weevils and caterpillars that cause major problems (feeding damage) in agro-ecosystems. Iridoviruses have also been found to infect insects that transmit plant pathogens and/or parasites of medical importance, such as mosquitoes, whiteflies and grasshoppers. We investigated the potential of iridoviruses for weevil control in hazelnut in Turkey. However, very little is known about insect iridoviruses, more specifically about gene function and regulation. Only two invertebrate iridoviruses have been sequenced, one of them infecting weevils and caterpillars, Chilo iridescent virus (CIV) (Jakob et al., Virology 2001, 286: 182-196). The latter study has shown that CIV encodes 468 open reading frames (ORFs) half of which are non-overlapping. There is little information on which of these ORFs encode proteins that are part of the virion structure, except the major capsid protein (MCP). We have carried out a proteomic analysis of CIV and have identified 55 proteins in the virus particle by SDS-PAGE and LC-MS/MS. These proteins range in size from 237 to 7 kDa, with peptide coverage up to 66%. Forty of these proteins were encoded by genes with orthologs in several other (fish) iridoviruses, as well as in ascoviruses. The remaining 15 proteins are, so far, unique for insect iridoviruses (CIV). This knowledge is important to determine which proteins are essential for the structure of CIV virions and which are involved in other aspects of the CIV infection process. Present work supported by The Scientific and Technological Research Council of Turkey (TÜB?TAK), grant number 2219. Proteomic analysis was carried out by Biqualys BV, The Advanced Analysis Company, Wageningen, The Netherlands.

Contributed Paper - Wednesday, 11:00 95

Analysis of MDSGHV transcripts in *Spodoptera frugiperda* cells and in house flies.

Tamer Salem - University of Florida, Gainesville, FL, USA; ***Alejandra Garcia- Maruniak*** - University of Florida, Gainesville, FL, USA; ***Verena-Ulrike Lietze*** - University of Florida, Gainesville, FL, USA; ***James Maruniak*** - University of Florida, Gainesville, FL, USA; ***Drion Boucias*** - University of Florida, Gainesville, FL, USA
Address for correspondence: nzs2000@gmail.com

A comprehensive study using 3'-RACE has revealed that 34 of the 108 Open reading frames (ORFs) described in *Musca domestica* salivary gland hypertrophy virus (MdSGHV) are transcribed in tandem; the upstream-ORF transcript reads through and co-terminates with the adjacent downstream ORF. Although the presence of tandem transcripts is common in different types of viruses, their role remains unclear. The high number of tandem transcripts in the MdSGHV indicates that this process may be important in MdSGHV regulation and/or in its infection cycle. Previous data has shown that MdSGHV084 was found as both a single and a tandem transcript that co-terminated with the adjacent MdSGHV085 (tMdSGHV084-085). *In vitro* and *in vivo* approaches were used to investigate the function and temporal transcription of these two ORFs. Transient expression of tMdSGHV084-085 in the absence and presence of viral infection was studied. The DNA region containing the MdSGHV084 and MdSGHV085 ORFs was cloned, and the resulting plasmid constructs were used to transfect *Spodoptera frugiperda* (Sf9) cells. To study the effects of virus on the transcription level of these ORFs, Sf9 cells were additionally co-transfected with mRNAs from infected house flies. The presence and abundance of both individual and tandem transcript in both treatments was assessed using reverse transcriptase PCR and quantitative real time polymerase chain reaction.

Contributed Paper - Wednesday, 11:15 96

Four major envelope proteins of white spot syndrome virus bind to form a complex.

Yipeng Qi - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, wuhan, Hubei, China; ***Qing Zhou*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, wuhan, Hubei, China; ***Yan Zhu*** - State Key Laboratory of Virology,

College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Feng Yan*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Xiamen, Fujian, China
Address for correspondence: qiyipeng@whu.edu.cn

Early events in white spot syndrome virus (WSSV) morphogenesis, particularly the formation of viral membranes, are poorly understood. The major envelope proteins of WSSV are VP28, VP26, VP24, and VP19. Our previous results indicated that VP28 interacts with VP26 and VP24. In the present study, we used coimmunoprecipitation assays and pull-down assays to confirm that the four major proteins in the WSSV envelope can form a multiprotein complex. Yeast two-hybrid assays were also used to test for interactions among the four proteins. In summary, three pairwise protein interactions (VP19-VP28, VP19-VP24, and VP24-VP26) and one self-association (VP24-VP24) were identified for the first time.

Contributed Paper - Wednesday, 11:30 97

Molecular analysis of protein kinase gene *(amv197)* of *Amsacta moorei* entomopoxvirus.

Hacer Muratoglu - Karadeniz Technical University, Trabzon, TR, Turkey; ***Remziye Nalcacioglu*** - Karadeniz Technical University, Trabzon, Turkey; ***Zihni Demirbag*** - Karadeniz Technical University, Trabzon, Turkey
Address for correspondence: zihni@ktu.edu.tr

Amsacta moorei Entomopoxvirus (AmEPV) appears to contain 279 open reading frames (ORFs) among which is the AMV197, composed of 900 nt and potentially encoding a protein of 299 amino acids. Sequence derived amino acid analysis of this ORF suggested it to be a serine / threonine protein kinase (PK) having conserved *pk* and serine / threonine *pk* domains. RT–PCR analysis of mRNA indicated that the transcription of the *pk* gene started 4 h post- infection (h p. i.) and continued to be expressed through 24 h p. i. Infection of Ld cells in the presence of Ara-C, followed by RT–PCR showed that *pk* is transcribed as an early gene. Transcription was initiated at 54 nt upstream of the translation start site. The vaccinia virus early promoter element G was also found at the correct position (-21) in the AmEPV *pk* gene. Rapid amplification of the 3'ends of the *pk* transcript showed that there are two polyadenylation start points. They are located at 22 and 32 nt's downstream of the translation stop site. Also, the translational stop site and poly (A) signal of pk are overlapped. The termination signal TTTTTGT sequence of vaccinia virus early genes was found just upstream of the 3' end of AmEPV *pk gene. Conserved amino acid subdomains of the AmEPV pk were found by sequence comparisons with pk's from other organisms. Analysis of the protein sequence of AmEPV pk gene reveals close identity with pk genes of other organisms. Delation of pk gene did not have any change on the replication of the virus in cell culture.*

Contributed Paper - Wednesday, 11:45 98-STU

The sfav1a p64 basic virion protein and its homologues comprise a novel family of viral genome condensing proteins.

Tatsinda Spears - Graduate Program in Cell, Molecular, and Developmental Biology, University of California, Riverside, Riverside, California, USA; ***Dennis K. Bideshi*** - California Baptist University, Riverside, California, USA; ***Jeffrey J. Johnson*** - Department of Entomology, University of California, Riverside, Riverside, California, USA; ***Yeping Tan*** - Department of Entomology, University of California, Riverside, Riverside, California, USA; ***Yves Bigot*** - Laboratorie d'Estude des Parasites Genetiques, 37200 Tours, France; ***Brian A. Federici*** - Department of Entomology, and Graduate Programs in Genetics and Cell, Molecular and Developmental Biology, University of California, Riverside, California, USA
Address for correspondence: tatsinda.spears@email.ucr.edu

Several ascoviruses (family Ascoviridae) are highly pathogenic to noctuid larvae. Ascovirus virions are large (130 by 200-400 nm) and have complex symmetry and structure. They consist of an inner particle composed of a protein-rich core that contains the dsDNA genome (100-186 kbp) that after assembly is enveloped to form the mature virion. An important yet unknown aspect of ascovirus biology is how their large genomes are condensed and packaged during virion assembly. Previously we showed that small basic histone, histone-like, and protamine-like proteins known to condense genomes of large dsDNA viruses like baculoviruses and poxviruses are absent in virions of the Spodoptera frugiperda ascovirus 1a (SfAV1a), the type species. Through studies of abundant SfAV1a virion proteins, we identified a novel highly basic DNA-binding protein, P64, that bound to its DNA, as well as to plasmid DNA. Two distinct domains not known to occur together in other proteins are present in P64. The aminoterminal domain contains 4 repeats of a virus-specific 2-cysteine adaptor motif potentially involved in protein-protein interactions, whereas the carboxy-terminal domain contains 14 tandem repeats of an arginine-rich motif that most likely binds to genomic DNA. Our recent studies suggested that P64's function is to condense the SfAV1a genome for encapsidation, as it is initially localized in virogenic stromae and then is progressively incorporated into the virion core. Here, using a combination of centrifugation and transmission electron microscopy techniques, we provide evidence demonstrating that P64 precipitates and condenses the SfAV1a genomic DNA. We also show that homologues of P64 also occur abundantly in the virions of other ascoviruses. These data support our hypothesis that P64 and its homologues compose a novel family of proteins that condense ascovirus genomic DNA beginning in virogenic stroma, and likely play a role in assisting its transport to the developing virion for encapsidation.

Contributed Paper - Wednesday, 12:00 99

Role of cellular microRNAs in ascovirus infection.

Mazhar Hussain - University of Queensland, Brisbane, Queensland, Australia; ***Sassan Asgari*** - University of Queensland, Brisbane, Queensland, Australia
Address for correspondence: s.asgari@uq.edu.au

MicroRNAs (miRNAs) are small non-coding RNAs that play significant roles in various cellular functions, including development, differentiation and immunity by regulating gene expression using a variety of mechanisms. They have been found to be widespread in living organisms, including viruses. There is accumulating evidence demonstrating the role of virus-encoded and cellular miRNAs in host-virus interactions influencing viral replication. Virus-encoded miRNAs have been shown to regulate expression of cellular as well as viral genes. On the other hand, cellular miRNAs have been described that affect expression of viral genes. Ascoviruses, transmitted by parasitoid wasps, cause a lethal infection in their lepidopteran hosts. Using *Heliothis virescens* ascovirus (HvAV3e) and a *Heliothis zea* fat body cell line (HzFB), we investigated differential expression of cellular miRNAs upon infection using miRNA microarray analysis. The chips contained 387 miRNA probes including miRBase insect miRNAs (Release 12) and custom miRNAs isolated from HzFB cells. Expression of several cellular miRNAs was up- or down-regulated upon infection with HvAV3e. One of the up-regulated miRNAs from HzFB cells (Hz-miR25) was found to be expressed later in infection and its expression coincided with a significant reduction in transcript

levels of its potential target genes in the viral genome (DNA dependent RNA polymerase and DdRp beta subunit). Further, overexpression of the miRNA in HzFB cells led to significant reduction in the target genes transcript levels whereas non-target genes were unaffected.

Contributed Paper - Wednesday, 12:15 100

Solenopsis invicta virus 3, a new positive-strand RNA virus infecting the red imported fire ant.

Steven Valles - USDA-ARS, Gainesville, Florida, USA
Address for correspondence: steven.valles@ars.usda.gov

The discovery of a new virus from the red imported fire ant is reported. Solenopsis invicta virus 3 (SINV-3) represents the third virus discovered from this ant species using the metagenomics approach. The positivestrand RNA, monopartite, bicistronic genome of SINV-3 was sequenced in entirety (Genbank accession number FJ528584), comprised of 10,386 nucleotides, and polyadenylated at the 3' terminus. The genome revealed 2 large open reading frames (ORFs) in the sense orientation with an untranslated region (UTR) at each end and between the two ORFs. RNA-dependent RNA polymerase (RdRp), helicase, and protease domains were recognized in ORF 1. SDS-PAGE separation of purified SINV-3 particles yielded 2 bands (ostensibly capsid proteins) with a combined molecular mass of 77.3 kDa which was similar to the mass predicted by ORF 2 (73.2 kDa). Phylogenetic analysis of the conserved amino acid sequences of the RdRp from dicistroviruses, iflaviruses, plant small RNA viruses, picornaviruses, and 4 unassigned positive-strand RNA viruses revealed a trichotomous phenogram with SINV-3 and Kelp fly virus comprising a unique cluster. Significant colony mortality was consistently observed in SINV-3-infected fire ants. The possibility of using SINV-3 as a microbial control agent for fire ants is discussed. 12:30 - 14:00

12:30 - 14:00 **STUDENT WORKSHOP & BOX LUNCH**
How to Get a Postdoc Position and Get Into the Scientific Network
Painted Horse I-II

Contributed Paper - Wednesday, 12:40 101

Life after the PhD: Find your own work-life balance.

Agata Jakubowska - University of Valencia, Burjassot, Valencia, Spain
Address for correspondence: agatajak@gmail.com

Among the most difficult decisions during the Ph.D. is what to do when one is finished with the thesis writing and dissertation. The typical career track in the academic life includes Ph.D., one or more post-doc positions, assistant and associate professor which finally lead to a full professorship. Of course there is always a way "out" of the academic campus straight to a wild industry world. When approaching the date of dissertation, a PhD-to-be has to make a decision to 'move up or move out'. In my presentation I am going to share my experience on how I made my choices after PhD defense and what are the possibilities for young researchers in Europe. I will discuss on how to find a balance between doing research and having a family, how to successfully finish the thesis and find a dream post-doc position and which questions one has to answer himself upon finishing the Ph.D. As one moves toward the last months of the Ph.D., she/he has to consider the full range of employment options. What we do directly after graduation will have a major impact on our professional progress. Evaluating all the options is a lot of work, but we have to allow time to do it properly and start well in advance.

Contributed Paper - Wednesday, 13:05 102

From passion to profession: An unconventional journey from high school teacher to research scientist.

Pasco B. Avery - UF/IFAS/Indian River Research and Education Center, Ft. Pierce, FL, USA
Address for correspondence: pbavery@ufl.edu

Most students follow the traditional career path: Bachelors, Masters, Ph.D., and then a post doctorate position. My path took a decades-long detour through high school classrooms. This presentation will describe how I maintained my interest and activities in the field of "real" science while teaching in a half-dozen different high schools in three different countries. Some of these activities were incorporated into my teaching; others were conducted outside of school. All of them contributed to my qualifications. Classroom activities included research projects and presentations conducted by whole classes and supervising independent studies by individual students. Outside activities included summers working in the field; earning a Ph.D. degree; and temporary research technician positions with the USDA, ARS.

Wednesday, 13:30 103

There's more than one way to skin a cat: Finding your path to a professorship.

Richard Plunkett - New Mexico Highlands University, Las Vegas, NM, USA
Address for correspondence: rplunkett@nmhu.edu

Good advice for faculty position interviewing abounds, but before the interview, being short-listed, before even beginning your search, there are hurdles to surmount and pitfalls to avoid. The current academic job search is extremely competitive and success requires more than just a curriculum vitae, a cover letter and a good suit. Tenacity, flexibility and imagination are vital components of finding the right postdoctoral experience or faculty job. My journey to a tenure-track position was circuitous, nontraditional, and supplied valuable lessons. I will describe my unorthodox experience and the insights it has provided regarding success in contemporary academia.

Symposium (Fungus Division) Wednesday, 14:00 - 16:00
Kokopelli Ballroom III

Insect Defense Responses to Fungal Pathogens

Moderators: Tariq Butt, Drion Boucias
Organizers: Tariq Butt, Rosalind James

Symposium - Wednesday, 14:00 104

Invertebrate antifungal immunity – Finding avenues for exploitation.

Miranda Whitten - Swansea University, Swansea, West Glamorgan, UK; ***Norman Ratcliffe*** - Swansea University, Swansea, West Glamorgan, UK; ***Tariq Butt*** - Swansea University, Swansea, West Glamorgan, UK; ***Drion Boucias*** - University of Florida, Gainesville, USA
Address for correspondence: pathos@ufl.edu

Invertebrates employ a variety of sophisticated innate humoral and cell-mediated immune strategies to cope with fungal infections, despite the absence of a fully adaptive immune system. Our understanding of these responses will enable a more targeted and efficient use of fungal biocontrol agents and assist protection strategies for beneficial species. Fungi must first breach physico-chemical barriers such as the exoskeleton

or gut wall. Rapid-response immune surveillance systems employing cell-surface recognition receptors and constitutively expressed circulating recognition proteins can then bind to and recognize fungal cell wall components. The detection of fungal infections relies not only on the recognition of characteristic cell wall components but also on virulence factors produced by the fungus. Together these trigger signaling cascades such as the Toll pathway, which activate immune cells or trigger the transcription of antimicrobial molecules and proteolytic cascades culminating in melanin deposition. Blood cells engage in phagocytosis or nodule formation, rapidly clearing the vast majority of invading organisms within hours, and these reactions may be accompanied by humoral melanotic encapsulation. Such basal, rapid-response reactions are augmented by the expression of a suite of inducible antimicrobial molecules. The possibility of immune specificity / memory, the exact role of proteolytic cascades culminating in melanin deposition, and the deployment of colony-wide social immunity strategies, are topics of renewed debate. The exciting picture emerging from ongoing research suggests a complex and exploitable crosstalk between immune, metabolic and reproductive pathways in invertebrates.

Symposium - Wednesday, 14:30 105

The *Drosophila melanogaster* model to study fungal and bacterial infections : Novel insights into insect host defense.

Marie Gottar - UPR 9022 du CNRS, Strasbourg, Alsace, France; ***Nadine Nehme*** - UPR 9022 du CNRS, Strasbourg, Alsace, France; ***Stefanie Limmer*** - UPR9022 du CNRS, Strasbourg; ***Samuel Liegeois*** - UPR9022 du CNRS, Strasbourg, France; ***Dominique Ferrandon*** - UPR9022 du CNRS, Strasbourg, Alsace, France; ***Alexei Matskevitch*** ; ***Jessica Quintin*** ; ***Richard Bou Aoun; Arshad Ayyaz; Philippe Giammarinaro; Shane Cronin; J. Andrew Pospisilik; Daniel Schramek; Ricardo de Matos Simoes; Ingo Ebersberger; Arndt von Haeseler; Josef Penninger***

Address for correspondence: D.Ferrandon@ibmc.u-strasbg.fr

Drosophila is a model for the study host defense against infections because of its powerful genetics. In a septic injury model, microbes are detected by Pattern Recognition Receptors (PRRs). These PRRs trigger in the fat body NF-kappaB signaling pathways: Toll (Gram-positive bacteria and fungi) and Immune Deficiency (Gram-negative bacteria). These pathways control the expression of hundreds of genes, including antimicrobial peptide genes such as Drosomycin, which kills filamentous fungi. Yeasts such as *Candida* do not kill wild-type flies but kill Toll pathway mutant flies. The Toll-dependent effector genes active on *Candida* remain to be identified. It is likely that other processes besides the systemic immune response concur to limit *Candida* infections. Using the entomopathogenic fungus *Beauveria bassiana*, we have discovered that a dual detection systems is used to sense fungal infections, namely a PRR (GNBP3)-dependent system, and the Persephone protease precursor that becomes activated into a functional protease by virulence factors of fungi such as PR1 from *Metarhizium anisopliae*. *B. bassiana* appears to have developed a strategy to elude detection by the PRR system, yet still activates the Toll pathway through Persephone. We have developed an intestinal infection model by feeding flies the potent pathogen *Serratia marcescens* (a few bacteria kill flies within a day in a septic injury model). In the oral infection model, however, the flies succumb in six days, likely as a result of gut damage and not because of septicemia, even though this Gram-negative bacterium is able to cross the digestive tract rapidly. Indeed, bacterial proliferation in the hemocoele is controlled by phagocytosis, and, as a result, the systemic immune response is not triggered. In contrast, the immune deficiency pathway is activated in the midgut and provides some protection against *S. marcescens*. To understand the infection from the vantage of the host, we have undertaken a genome-wide screen of the Vienna Drosophila RNAi Center collection to identify lines that present an enhanced or decreased survival. We have screened about 75% of the *Drosophila* genome by decreasing ubiquitously their expression. In secondary screens, we have tested our hits by decreasing their expression in either the midgut or in hemocytes. We show that the JAK-STAT pathway is required to drive intestinal stem cell proliferation induced by the infection, likely to compensate the extensive cell death of enterocytes observed in the midgut epithelium. While the exact function in host defense of many of the genes identified in this screen remains presently unknown, our data indicate that host defense involves many processes that are not limited to classical innate immune response pathways as exemplified here by the role of the JAK/STAT pathway in the regulation of epithelial homeostasis in response to infection.

Symposium - Wednesday, 15:00 106

Transcriptome analysis of honey bee, *Apis mellifera* larvae infected with chalkbrood fungus.

Katherine Aronstein - USDA/ARS, Weslaco, TX, USA; ***Dan Murray*** - USDA/ARS, Weslaco, TX, USA

Address for correspondence: Kate.Aronstein@ars.usda.gov

In this study, we present the experimental identification of gene transcripts that are altered in response to infection of honey bee, *Apis mellifera* larvae with fungal pathogen, *Ascosphaera apis*. Using the cDNA-AFLP approach, we determined a large number of transcriptional products that varied in abundance within 24 hours post infection in comparison to control. A selected panel of the differentially expressed genes were cloned and sequenced followed by quantitative real-time RT-PCR (qRT-PCR) analysis in a wider range of samples and collection time points. Honey bee transcripts identified in this study were involved in critical functions related to gene transcriptional regulation, apoptotic degradation of ubiquitinated proteins, nutritional regulation, pathogen recognition and signaling. A number of genes were identified that are involved in general mechanisms of stress adaptation. The most significant finding is the identification of the chitinase-like enzyme that breaks down glycosidic bonds in chitin and potentially implicated in anti-fungal activity. We have also utilized qRT-PCR approach to probe whether some elements of the Toll pathway and anti-microbial peptides (AMPs) were responsive to fungal infection. The implications of these findings are discussed.

Wednesday, 15:30 107

Immunity related genes expressed in the alfalfa leafcutting bee, *Megachile rotundata.*

Junhuan Xu - Utah State University, Department of Biology, Logan, Utah, USA; ***Rosalind James*** - USDA-ARS, Pollinating Insects Biology, Management and Systematics, Logan, Utah, USA

Address for correspondence: xu@biology.usu.edu

Bees are a large, diverse group of insects that collect pollen and nectar from plants to feed to their young, and as a result, play an important ecological role as pollinators. Honey bees, which form large colonies with a complex social structure are probably the most well known bee, but the majority of species are actually solitary, with little or no social structure. It has been proposed that social insects are more vulnerable to disease outbreaks due to the density and frequent interactions among individuals in a nest, and as a result, may have evolved special mechanisms for evading disease, including specialized immune responses, but such hypotheses cannot be tested when we know virtually nothing about the immune response of solitary bees. Using an EST database for both healthy and chalkbrood-

infected larvae of a solitary bee, the alfalfa leafcutting bee, we identified expression of 104 genes known to have immunity-related functions in other insects. Other putative immune response genes were identified in this solitary bee using PCR amplification with primers designed for honey bee immune response genes. The genes we isolated and identified code for proteins with a wide variety of innate immune responses, including pathogen recognition, phagocytosis, the prophenoloxidase cascade, melanisation, coagulation and several signaling pathways. The data on innate immunity of alfalfa leafcutting bee will provide us a basic model for our further analysis of gene expression patterns in response to chalkbrood disease.

CONTRIBUTED PAPERS Wednesday, 14:00 - 16:00
White Pine I-II

Microbial Control II

Moderator: Steven Arthurs

Contributed Paper - Wednesday, 14:00 108

Compatibility of *Bacillus thuringiensis* Cry1 and Vip3a proteins for resistance management in *Spodoptera frugiperda*.

Janete A.D. Sena - University of Valencia, Burjassot, Valencia, Spain; ***Carmen Sara Hernández-Rodríguez*** - University of Valencia, Burjassot, Valencia, Spain; ***Juan Ferré*** - University of Valencia, Burjassot, Valencia, Spain
Address for correspondence: juan.ferre@uv.es

Widespread adoption of Bt crops may eventually select for insect resistance, threatening the efficacy of these biotechnological products. For this reason, it is desirable to combine two of more toxins with different mode of action in the same plant. *Bacillus thuringiensis* produces, besides the insecticidal proteins which accumulate in the parasporal crystal (Cry and Cyt proteins), secreted insecticidal proteins produced during the vegetative growth (Vip proteins). The main goal of the present study was to determine the possible interactions, at the binding site level, of two Vip3A proteins (Vip3Aa1 and Vip3Af1) and two Cry1 proteins (Cry1Ab and Cry1Fa) in *S. frugiperda*. Bioassays carried out with neonate larvae showed that the two Vip3A proteins were more toxic than the Cry1 proteins tested. LC_{50} values (mortality scored after 7 days) were 21, 49, 170, and 870 ng/cm2 for Vip3Af1, Vip3Aa1, Cry1Fa and Cry1Ab, respectively. Binding assays with BBMV (brush border membrane vesicles) were performed with radiolabeled Cry1Ab and biotinylated Cry1Fa and Vip3Af1. Heterologous competition experiments revealed the occurrence of independent specific binding sites for Cry1 and Vip3A proteins. Cry1Ab and Cry1Fa competed for the same binding sites, whereas Vip3Aa competed for those of Vip3Af1. From a resistance management perspective, pyramiding of either Cry1Ab or Cry1Fa with either Vip3Aa1 or Vip3Af1, in the same plant, is highly recommended for a better and long lasting control of *S. frugiperda*.

Contributed Paper - Wednesday, 14:15 109-STU

Identification and characterization of *Bacillus* species toxic to several mosquito species.

Sabrina Hayes - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University, Panama City, FL, United States of America; ***Hyun-Woo Park*** - John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University, Panama CIty, Fl, USA; ***Michael Hudon*** - Indian River Mosquito Control District, Vero Beach, Fl, USA
Address for correspondence: sabrina.hayes@famu.edu

In an effort to find novel mosquitocidal bacteria for controlling vector mosquitoes, VB17 and VB24 were isolated from dead mosquito larvae collected in Vero Beach, Florida. VB 17 and VB 24 were identified as new *Bacillus* species through the use of gas chromatographic analysis of fatty acids methyl esters (GC-FAME) and by 16s rRNA gene sequence alignment. The closest *Bacillus* species known for VB17 and VB24 were *Bacillus badius,* and *Bacillus sphaericus* and *Bacillus fusiformis,* respectively. Spores of VB17 had peculiar hair-like extension all around them and some sporulated cells of VB17 produced an amorphous inclusion that might be mosquitocidal. SDS-PAGE of the sporulated cultures of VB17 and VB24 indicated they have major proteins with high molecular sizes. Bioassay results showed that VB17 is toxic to *Aedes taeniorhynchus* (LC50 = 1.3 ng/ml), *Culex quinquefasciatus* (LC50 = 6.1 ng/ml), and *Anopheles quadrimaculatus* (LC50= 6.4 ng/ml). VB24 is toxic to *Ae. taeniorhynchus* (LC50 = 4.6 ng/ml), *Cx. quinquefasciatus* (LC50 = 6.7 ng/ml) and *An. quadrimaculatus* (LC50 = 6.0 ng/ml). Interestingly, none of these isolates were active against *Aedes aegypti.* Both isolates showed significantly higher toxicity against mosquito species in comparison to Bs 2362, suggesting that they may be good alternatives for controlling several mosquito species.

Contributed Paper - Wednesday, 14:30 110-STU

The dose-transfer chain: improving the lab-to-field process in cocoa.

Nick Jessop - Imperial College, Ascot, Berkshire, UK; ***Godfred Awudzi*** - Cocoa Research Institute of Ghana, Eastern Region, Ghana; ***Roy Bateman*** - Imperial College, Ascot, Berkshire, UK
Address for correspondence: nick.jessop@imperial.ac.uk

West Africa produces approximately 70% of the World's cocoa, with Ghana and the Côte D'Ivoire alone accounting for >50%. In Ghana it is estimated that 40% of the GDP is derived from the cocoa industry, which also provides nearly half of all employment. Recent studies have shown that 25-30% of cocoa acreage in Ghana suffers from significant damage from two major insect pests, Distantiella theobromae and Sahlbergella singularis (Heteroptera: Miridae). Our project, funded by Cocoa Research UK, aims to develop a mycoinsecticide for use in the control of cocoa mirids. Application of pesticides to cocoa is achieved using a combination of motorised mistblowers, originally developed for mirid control, and side lever knapsack sprayers (which are more often used by smallholders against black pod disease). We describe possible improvements to target uptake dose transfer process of contact pesticides, including mycoinsectides, having obtained quantitative data using motorised mistblowers and pods as a model. In anticipation of a suitably virulent isolate we have focused on the question of "how can virulence in the laboratory be transformed to efficacy in the field". Laboratory and field data will be presented to elucidate likely dose-transfer mechanisms for secondary pick up of conidia by mirids, using a novel tracer surrogate and quantitative analysis of formulation uptake.

Contributed Paper - Wednesday, 14:45 111-STU

Utilization of entomopathogenic fungi for the control of plant pathogenic fungi.

Sastia Prama Putri - International Center for Biotechnology, Osaka University, Suita, Osaka, Japan; ***Hiroshi Kinoshita*** - International Center for Biotechnology, Osaka University, Suita, Osaka, Japan; ***Fumio Ihara*** - National Institute of Fruit Tree Science, Morioka, Iwate, Japan; ***Yasuhiro Igarashi*** - Biotechnology Research Center, Toyama Prefectural

University, Imizu, Toyama, Japan; ***Takuya Nihira*** - International Center for Biotechnology, Osaka University, Suita, Osaka, Japan
Address for correspondence: sastia_prama_putri@icb.osaka-u.ac.jp

Entomopathogenic fungi have been often considered solely as insect pathogens, while their other roles in nature are mainly unknown. Since these fungi are known to produce various metabolites during infection and proliferation in insects, entomopathogenic fungi can be a potential source of novel bioactive compounds. This work describes the first systematic study on the antifungal activity of 420 entomopathogenic fungi isolated in Japan against two economically important plant pathogenic fungi, namely *Phytophthora sojae* P6497 and *Aphanomyces cochlioides* AC-5. The result of dual culture assay showed that 67.3% of the tested isolates displayed inhibitory activity against at least one of the plant pathogens, indicating that the highly active entomopathogenic fungal strains could be used as biocontrol agents against plant diseases caused by *P. sojae* and *A. cochlioides*, in addition to their current use against agriculturally important pests. During the profiling of antifungal activity of entomopathogenic fungi against the plant pathogens, we isolated a new maleimide compound, farinomalein from *Paecilomyces farinosus* HF599 that displayed a potent inhibitory activity (5 ?g/disk) against *P. sojae*. The isolation of the new antifungal compound from entomopathogenic fungi further raised the prospect of using insect fungal pathogens as bioresources of novel compounds for the control of soil-borne plant pathogens.

Contributed Paper - Wednesday, 15:00 112

Characterization of *Beauveria bassiana* isolates associated with *Agrilus planipennis* populations in Michigan.

Louela Castrillo - Cornell University, Ithaca, NY, USA; ***Leah Bauer*** - USDA Forest Service, East Lansing, MI, USA; ***Houping Liu*** - Michigan State University, East Lansing, MI, USA; ***Michael Griggs*** - USDA ARS, Ithaca, NY, USA; ***John Vandenberg*** - USDA ARS, Ithaca, NY, USA
Address for correspondence: jdv3@cornell.edu

In Michigan, a survey of fungal entomopathogens of the emerald ash borer (EAB), a major invasive pest of ash trees, resulted in the isolation of *Beauveria bassiana* strains from late-instar larvae and pre-pupae. These strains were characterized and compared to ash bark- and soil-derived isolates to determine their potential sources and to reveal how immature EAB become infected with fungi. Genetic characterization using seven microsatellite markers showed that most of the EAB-derived strains clustered with bark- or soil-derived strains collected from the same site, indicating the indigenous nature of most strains isolated from EAB. The number of *B. bassiana* colony forming units was higher from soil than from bark samples, suggesting that soil may serve as the primary reservoir for fungal inocula. These inocula may be carried by rain splash and air current from the soil to the lower tree trunk, where EAB may become infected. Additionally, inocula could come from infected EAB or other insects infesting ash trees. Bioassays of five representative strains showed three with comparable virulence to the commercial strain GHA, causing >60% mortality in EAB adults within five days after exposure by dipping in 106 conidia/ml. These data demonstrate that indigenous strains of *B. bassiana* have potential for use as control agents against EAB and suggest that fungal inocula applied to ash trunks may prove viable for controlling EAB in the field.

Contributed Paper - Wednesday, 15:15 113-STU

Storage conditions affect intensity of delayed germination in *Beauveria bassiana* and *Metarhizium anisopliae* conidia.

Marcos Faria - Cornell University, Ithaca, NY, USA; ***Mark Ramos*** - USDA-ARS, Ithaca, NY, USA; ***Ann Hajek*** - Cornell University, Ithaca, NY, USA; ***Stephen Wraight*** - USDA-ARS, Ithaca, NY, USA
Address for correspondence: steve.wraight@ars.usda.gov

We report on causes of a phenomenon previously noticed among entomopathogenic fungi following long-term storage, in which some conidia do not promptly initiate germination upon encountering a suitable substrate. Comparative experiments with *Beauveria bassiana* (Bb) and Metarhizium anisopliae (Ma) revealed that conidia stressed by exposure to various storage conditions germinated slowly when inoculated directly onto yeast-extract agar, but were able to germinate rapidly (within 24 h at 25 ºC) following a preliminary period of slow-hydration under high humidity conditions. In-depth studies with Bb demonstrated that the proportion of conidia undergoing delayed germination (requiring > 24 h for germination) increased with increasing water activity (aw) during storage. Measurements of O2/CO2 concentrations in storage containers revealed that increased water activity was associated with increased respiration. Levels of delayed germination were also affected by storage temperature. In a two-month time-course study, the proportion of Bb conidia expressing delayed germination was < 3% at 25 ºC, 9% at 40 ºC, and 55% at 50 ºC (aw held constant). Lower overall viabilities were associated with higher proportions of slow-germinating conidia. In conidial samples with 70¬–85% overall viability following storage at 50 ºC for 34 days, ca. 20–80% of conidia exhibited slow germination, with proportions increasing with increasing O2 concentrations and water activities. The lowest rate of delayed germination was observed at 0.3% O2 and 0.100 aw (the minimum levels of each factor tested). The results of these studies strongly suggest that conidia exhibiting delayed germination are effectively in a moribund state. Laboratory bioassays with beet armyworm larvae indicated that moribund conidia were significantly less virulent than normal conidia. In order to minimize stress, storage of mycoinsecticides in packages designed to maintain an oxygen-free, low aw environment and avoidance of prolonged exposure to abusive temperatures are highly desirable strategies.

Contributed Paper - Wednesday, 15:30 114

Production of microsclerotia of *Metarhizium anisopliae* using deep-tank, liquid fermentation.

Mark Jackson - USDA-ARS-NCAUR, Peoria, Illinois, USA; ***Stefan Jaronski*** - USDA-ARS-PARL, Sydney, Montana, USA
Address for correspondence: mark.jackson@ars.usda.gov

The entomopathogenic fungus *Metarhizium anisopliae* is a pathogen of numerous soil-dwelling insects and has been registered in the United States and other countries as a microbial pest control agent. Recent studies using various strains of *M. anisopliae* showed that small sclerotia (microsclerotia) were produced in high concentrations in liquid culture under the appropriate nutritional and cultural conditions. Granules containing microsclerotia of *M. anisopliae* were desiccation tolerant, germinated sporogenically following rehydration, and, when soil incorporated, infected and killed the sugar beet root maggot, *Tetanops myopaeformis*. In this study, we evaluated the potential for using deep-tank fermentation for the large-scale production of microsclerotia of *M. anisopliae* F52. Sixteen 100L deep-tank fermentations of *M. anisopliae* were conducted using glucose as the carbon source and acid hydrolyzed casein as the nitrogen source. Mean values for biomass and microsclerotia yields were 20.9 g L^{-1} and 6.4 x 10^7 microsclerotia L^{-1}, respectively. A diatomaceous earth (DE) filter aid was added to the fermentation broth containing microsclerotia of *M. anisopliae* and the

fungal biomass was separated from the culture supernatant using a rotary drum vacuum filter. Dewatered microsclerotia – DE preparations were granulated and air-dried to approximately 2.5% moisture. When rehydrated on water agar and incubated at 28 ºC for one day, virtually all microsclerotia – DE granules (97-100%) germinated hyphally and, after 8 days incubation, mean conidia production was 1.1 x 10^9 conidia $gram^{-1}$ dried microsclerotial preparation. Following storage for one year under vacuum in polyethylene bags at 4 ºC, the mean value for conidia production by air-dried microsclerotia – DE preparations was 5.8 x 10^8 conidia g^{-1}. Fermentor-produced microsclerotial granules were highly efficacious against sugarbeet root maggot in soil-based lab bioassays. Our results suggest that deep-tank fermentation is a promising method for the large-scale production of stable microsclerotia of *M. anisopliae*.

Contributed Paper - Wednesday, 15:45 115

Influence of carbohydrates on the control efficacy of the entomopathogenic fungus *Beauveria bassiana*.

Jeong Jun Kim - Applied Entomology Division, Suwon, Gyeonggi, Republic of KOREA ; ***Seon Heo*** - Applied Entomology Division, Suwon, Gyeonggi, Republic of KOREA ; ***Yujin Song*** - Applied Entomology Division, Suwon, Gyeonggi, Republic of KOREA ; ***Chang-Gyu Park*** - Applied Entomology Division, Suwon, Gyeonggi, Republic of KOREA ; ***Hong-Hyun Park*** - Applied Entomology Division, Suwon, Gyeonggi, Republic of KOREA
Address for correspondence: jjkim@rda.go.kr

Aphids are serious insect pests in greenhouse cultivated vegetables. The rapid population increase and the mixed developmental stages of aphid populations make microbial control difficult. The short developmental period of each instar contributes to reduced mortality because short molting periods can remove spores on the cuticle before germination and infection can occur. Therefore, rapid spore germination could increase control efficacy of entomopathogenic fungi. We investigated germination stimulants of *Beauveria bassiana* isolate 11218 conidia. This isolate has high pathogenicity against green peach aphid in Korea. Five concentrations of various carbohydrates were mixed with conidia of *B. bassiana* and incubated on 1.5% water agar for 12 hours. Carbohydrate concentration did not affect germination. Fructose, mannose and skim milk stimulated spore germination compared to control. At 0.5%, the lowest concentration, spore germination was 76 ± 9.0% in fructose, 52 ± 8.4% in mannose, 50 ± 7.1% in skim milk, 39 ± 2.9% in glucose and 33 ± 8.3% in control. Bioassays against cotton and green peach aphids were conducted with conidia suspended in 0.5% fructose, mannose, skim milk and glucose. Spore suspensions with mannose and fructose induced the highest mortalities among the 4 carbohydrates tested.

CONTRIBUTED PAPERS Wednesday, 14:00 - 16:00
Kokopelli Ballroom II

Virus III

Moderators: Basil Arif, Zhihong Hu

Contributed Paper - Wednesday, 14:00 116

Defining the response of *Mamestra brassicae* to mixed infections.

Helen Hesketh - NERC Centre for Ecology and Hydrology, Oxford, Oxfordshire, United Kingdom; ***Claus Svendsen*** - NERC Centre for Ecology and Hydrology, Wallingford, Oxfordshire, United Kingdom; ***Rosie Hails*** - NERC Centre for Ecology and Hydrology, Oxford, Oxfordshire, United Kingdom
Address for correspondence: hhesketh@ceh.ac.uk

The analysis of multiple dose-response assays usually focuses on overall response patterns of synergism or antagonism. It is rare that more complex response patterns are described that incorporate dose-level or dose-dependent specific synergism and antagonism. We will present an example where we have adapted models from recently developed ecotoxicological mixture dose-response analysis to specifically describe the mortality response of cabbage moth larvae *Mamestra brassicae* exposed to combinations of pathogens and toxins. This forms part of a larger study investigating whether baculoviruses can be combined with other entomopathogens to achieve improved biological control of insect pests. Larvae of *M. brassicae* were exposed in the laboratory to a closely related nucleopolyhedrovirus *Panolis flammea* NPV (*Pafl*NPV) or a homologous baculovirus *Autographa californica* NPV (*Acal*NPV) in mixtures with either *Bacillus thuringiensis* subsp. *kurstaki (Btk),* the pesticide Spinosad (active ingredients Spinosyns A & D) or the chemical Diethyldithiocarbamic acid (DETC) a sodium salt which has known suppression effects of the host immune system. To account for the antifeedant activity of *Btk* and Spinosad, the pathogen concentration received by each larva was adjusted relative to food consumption. Mortality of larvae due to each pathogen/toxin was assessed in each assay for 64 treatment combinations. Both *Pafl*NPV and *Acal*NPV interacted in a similar way in mixtures with *Btk* and displayed a significant level of synergism across several of the doses tested. The interaction between Spinosad/*Pafl*NPV and DETC/*Pafl*NPV was more complex but was described by the adapted ecotoxicology independent action model. The use of such models enables us to identify doses at which synergy with another pathogen or toxin can increase baculovirus mortality in a Lepidopteran host.

Contributed Paper - Wednesday, 14:15 117-STU

Mixed infections of wild-type and fast-acting recombinant variants in insect-baculovirus pathosystems.

Liljana Georgievska - Wageningen University, Laboratory of Virology, Wageningen, Gelderland, The Netherlands; ***Wopke van der Werf*** - Wageningen University, Centre for Crop Systems Analysis, Wageningen, The Netherlands; ***Mark P. Zwart*** - Wageningen University, Laboratory of Virology, Wageningen, The Netherlands; ***Kelli Hoover*** - Pennsylvania State University, Department of Entomology, PA, USA; ***Jenny Cory*** - Department of Biological Sciences, Canada; ***Just M. Vlak*** - Wageningen University, Laboratory of Virology,
Address for correspondence: liljanag@iastate.edu

Mixed infections of baculoviruses in insect hosts are quite common in nature. This leads to 'within-host' and 'between-host' competition between virus variants. Because both levels of selection will contribute to overall biological fitness, both must be included in assessments of the fitness of fast-acting recombinant baculoviruses. We investigated baculovirus fitness parameters in single and mixed infection of insect larvae, in single and serial passage experiments in lepidopteran hosts (*Helicoverpa armigera*, *Spodoptera exiqua* and *Trichoplusia ni*) in laboratory, greenhouse and field settings. Median time to death in third instar larvae of *H. armigera* (Hübner) was lower in insects challenged with a mixture of wild type (HaSNPV-wt) and mutant (?egt, HaSNPV-LM2) *Helicoverpa armigera* SNPV, than in larvae infected with only HaSNPV-wt. The results from a behavioral study on cotton (greenhouse, field) indicated that the transmission of HaSNPV-LM2 is not modified by the absence of the egt gene, whereas in the case of the HaSNPV-AaIT (?egt, + AaIT) lower virus yield as well as altered caterpillar behavior could compromise virus fitness. Virus transmission in greenhouse and

field was not reduced, when HaSNPV-LM2 was used in mixed infections with HaSNPV-wt. However, a reduction of 'between host' transmission was recorded when *H. armigera* larvae were co-infected with HaSNPV-wt and HaSNPV-AaIT. Serial passage experiments with *S. exigua* and *T. ni* showed positive selection for wild type SeMNPV and AcMNPV over genetically modified variants (?egt, + AaIT in the case of SeMNPV, and ?egt in the case of AcMNPV) over passages. These findings can help to understand long-term dynamics of virus genotypes in virus-insect-host plant systems. They can also help foresee potential consequences of the introduction of genetically-modified or exotic baculoviruses in agro-ecosystems.

Contributed Paper - Wednesday, 14:30 118

Expression of *Helicoverpa armigera* chitin deacetylase-like protein improves nucleopolyhedrovirus speed of kill.

Agata Jakubowska - University of Valencia, Burjassot, Valencia, Spain; ***Silvia Caccia*** - University of Valencia, Burjassot, Valencia, Spain; ***Karl Gordon*** - CSIRO Entomology, Canberra, Australia; ***Juan Ferré*** - University of Valencia, Burjassot, Valencia, Spain; ***Salvador Herrero*** - University of Valencia, Burjassot, Valencia, Spain
Address for correspondence: agatajak@gmail.com

Insect midgut is the main site for the interaction between insects and their pathogens. Most insect pathogens, like bacteria, viruses and protozoa, are usually taken up orally, with food. In arthropods, the gut is lined with the peritrophic membrane (PM), which serves as the first line of defence and is composed of chitin fibrils and proteins. In the present work we describe a midgut protein, HaCDA5a, from the cotton bollworm, Helicoverpa armigera. An expressed sequence tag (EST) with homology to chitin deacetylase-like protein was selected from a group of *H. armigera* genes down-regulated after infection with H. armigera single nucleopolyhedrovirus (HaSNPV). HaCDA5a sequence analysis cofirmed the presence of a complete chitin deacetylase domain (CDA), but so far no chitin deacetylase activity was detected. Instead we have shown strong binding of HaCDA5a to chitin, while no chitin binding domain, as described for all the currently known PM proteins, was present. The presence of HaCDA5a in insect PM together with its down-regulation after pathogen infection led us to hypothesize that this protein may change the permeability of PM, by its deacetylation or other enzymatic activity allowing easier pathogen penetration. To test this hypothesis we constructed a recombinant nucleopolyhedrovirus to express HaCDA5a in insect cells and tested its influence on PM permeability as well as the influence of HaCDA5a overexpression on the performance of baculovirus. The experiments showed the increase in PM permeability due to HaCDA5a treatment. Bioassays on *Spodoptera exigua* and *Spodoptera frugiperda* larvae showed that administered orally NPV expressing HaCDA5a is more virulent that the virus non-expressing this protein, however there is no change in virulence when both viruses are injected intrahaemocoelically. These findings confirm the hypothesis that HaCDA5a is most probably acting on PM and revealed the possibility of baculovirus improvement by host gene manipulation.

Contributed Paper - Wednesday, 14:45 119

Tissue tropism of the *Musca domestica* salivary gland hypertrophy virus.

Verena-Ulrike Lietze - Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; ***Tamer Z. Salem*** - Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; ***Pannipa Prompiboon*** - Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; ***Drion Boucias*** - Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA
Address for correspondence: vlietze@ufl.edu

A quantitative real-time PCR assay was developed to investigate the tissue tropism of the *Musca domestica* salivary gland hypertrophy virus (MdSGHV) in infected adult house flies. At 3 d post-infection, hemolymph samples were collected, and separate pools of salivary glands, midguts, ovaries, abdominal fat body, crops, air sacs and brains were dissected into Tri-Reagent and subjected to DNA and RNA isolation. Healthy flies served as negative controls. The presence and quantity of viral DNA and transcripts in these samples were assessed by specific primer sets targeting five different open reading frames (ORFs) representing structural and non-structural genes of the MdSGHV genome. The *M. domestica* ribosomal 28S gene served as a reference gene. Results showed that viral DNA was present in all samples collected from infected females. Average numbers of viral gene copies per 50 ng of DNA ranged from 6.2×10^5 in hemolymph to 6.2×10^8 in salivary glands. Accordingly, transcription levels were lowest in the hemolymph and highest in the salivary glands. High levels of MdSGHV transcription were also found in fat body, air sacs and brain samples. Transcript abundance within each sample type varied slightly between the five ORFs examined, but these differences were not significant. It should be noted that all of the examined organ samples likely contained trachea. The DNA presence and high transcriptional activity of the MdSGHV in all of these organs indicate a significant role of the tracheal system in the systemic spread of the virus within the infected fly.

Contributed Paper - Wednesday, 15:00 120

Geographical distribution of *Musca domestica* salivary gland hypertrophy virus that infects and sterilizes female house flies.

Pannipa Prompiboon - University of Florida, Gainesville, Florida, USA; ***Verena- Ulrike Lietze*** - University of Florida, Gainesville, Florida, USA; ***Tamer Salem*** - University of Florida, Gainesville, Florida, USA; ***Drion Boucias*** - University of Florida, Gainesville, Florida, USA
Address for correspondence: pannipa_mu@yahoo.com

The house fly, *Musca domestica* L. (Diptera: Muscidae), is a cosmopolitan insect and known to be an economically important pest of livestock and poultry. Populations of *M. domestica* are naturally infected with a salivary gland hypertrophy virus (MdSGHV), a non-occluded dsDNA virus that completely inhibits egg production in infected females and is characterized by hypertrophic salivary gland symptoms. To date, MdSGHV has been detected in house fly samples from North America, Europe, Asia, the Caribbean and the Southwestern Pacific. In this study, collected house fly samples were dissected to observe salivary gland hypertrophy symptoms, and the infected glands were collected for MdSGHV isolation and amplification in lab reared house flies. Geographic differences among the isolated MdSGHVs were examined by molecular and biological approaches. Approximately 600-bp nucleotide sequences from each of five open reading frames encoding DNA polymerase and four *per os* infectivity factor proteins (pif-1, p74, pif-2 and pif-3) were selected for phylogenetic analyses. Nucleotide sequences from 15 different geographic isolates showed polymorphism that correlated with geographic source. The virulence of these geographic MdSGHV isolates was evaluated by *per os* treatments of newly emerged and one-day-old house flies. Results showed that infectivity varied among the different isolates and different aged flies tested. The variation observed in biological activity does not correspond to genotypic variation.

Contributed Paper - Wednesday, 15:15 121

Strategies to control salivary gland hypertrophy virus (SGHV) infection in tsetse laboratory colonies.

Adly Abd-Alla - Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Vienna, Austria, Austria; ***Andrew Parker*** - Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Vienna, Austria; ***Max Bergoin*** - Laboratoire de Pathologie Comparée, Université Montpellier II, France, Montpellier, France; ***Just Vlak*** - Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands, Netherlands; ***Marc Vreysen*** - Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Vienna, Austria
Address for correspondence: a.m.m.abd-alla@iaea.org

The successful elimination of a population of the tsetse fly *Glossina austeni* from Unguja Island (Zanzibar) using an area-wide integrated pest management approach with a sterile insect technique (SIT) component (1994-1997), encouraged several African countries to include SIT in their national tsetse control programs. A large facility to produce tsetse flies for SIT application in Ethiopia was inaugurated in 2007. To support this project, the Entomology Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria successfully established a Glossina pallidipes tsetse colony originating from Ethiopia in 1996, but 85% of adult flies displayed symptoms of Salivary Gland Hypertrophy (SGH). As a result, the colony declined and became extinct by 2002. SGH is caused by the *G pallidipes* salivary gland hypertrophy virus (GpSGHV) which reduces the fecundity of infected flies. SGHV is also present in the *G. pallidipes* colony that originates from Uganda and which has been maintained at the Entomology Unit in Seibersdorf for the last 20 years. The prevalence of symptomatic infection in this colony is stable around 10%. The difficulties experienced with the rearing of *G. pallidipes*, epitomized by the collapse of the *G. pallidipes* colony originating from Ethiopia, indicates the urgent need for a GpSGHV management strategy. Different approaches to prevent virus replication and its horizontal transmission during blood feeding have been attempted and their preliminary results will be presented. These include adding to the blood diet antiviral drugs such as acyclovir and valacyclovir or antibodies against SGHV virion proteins. In addition, preliminary attempts to silence the expression of this protein using RNA interference are described. This work was done in collaboration with the following persons and they are co-authors: Patrick Abilaa, Henry Kariithia,c, François Cousseransb, Abd el Naser Elashryd.

Wednesday, 16:30 - 17:30
Kokopelli Ballroom I

POSTERS 2

Posters should be displayed from Wednesday 8:00 to Thursday 13:30.

BENEFICIAL INVERTEBRATES

Poster/Beneficial Invertebrates - Monday, 16:30 - 17:30 BI-1

Hytrosaviridae: A proposal for classification and nomenclature of a new insect virus family.

Adly Abd-Alla - Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Vienna, Austria, Austria
Address for correspondence: a.m.m.abd-alla@iaea.org

Salivary gland hypertrophy viruses (SGHVs) have been identified from different dipteran species, such as the tsetse fly *Glossina pallidipes* (GpSGHV), the house fly *Musca domestica* (MdSGHV) and the narcissus bulbfly *Merodon equestris* (MeSGHV). These viruses share the following characteristics: (i) they produce non-occluded, enveloped, rod-shaped virions that measure 550-1000 nm in length and 80-100 nm in diameter; (ii) they possess a large circular double-stranded DNA (dsDNA) genome ranging in size from 120-190 kbp and having G+C ratios ranging from 28-44%; (iii) they cause overt salivary gland hypertrophy symptoms in dipteran adults and partial to complete sterility. The available information on the complete genome sequence of GpSGHV and MdSGHV indicates significant co-linearity between the two viral genomes, whereas no co-linearity was observed with baculoviruses, ascoviruses, entomopoxviruses, iridoviruses and nudiviruses, other large invertebrate DNA viruses. The DNA polymerases encoded by the SGHVs are of the type B and closely related, but are phylogenetically distant from DNA polymerases encoded by other large dsDNA viruses. The great majority of SGHV ORFs could not be assigned by sequence comparison. Phylogenetic analysis of conserved genes clustered both SGHVs, but distantly from the nudiviruses and baculoviruses. On the basis of the available morphological, (patho)biological, genome and phylogenetic data, we propose that the two viruses are members of a new virus family named Hytrosaviridae. This family currently comprises three unassigned species GpSGHV, MdSGHV and MeSGHV. Here, we present the characteristics and the justification for establishing this new virus family. This work done by the Hytrosaviride study group members: J. M. Vlak, M. Bergoin, J. E. Maruniakd, A. Parkera, J. P. Burand, J. A. Jehle, and D. G. Boucias and the are co-authors.

Poster/Beneficial Invertebrates - Monday, 16:30 - 17:30 BI-2

Bee cell cultures: (*Apis mellifera,* Apidae: Hymenoptera.)

Wayne Hunter - USDA,ARS, Fort Pierce, Florida, USA
Address for correspondence: wayne.hunter@ars.usda.gov

To meet the critical needs of apiculture research in addressing Colony Collapse Disorder, CCD, bee cell lines were established. The importance of bees as pollinators and the recent emergence of CCD, has brought the need for bee cell cultures to the forefront of the research community. Bee cell lines were established using the Hert-Hunter- 70, HH70, insect medium developed for psyllid cell lines. These cell lines provide a means to isolate individual virus strains for further evaluation of the impact and role of Israeli Acute Paralysis virus, IAPV, and the other ssRNA viruses known to infect bees and other Hymenoptera.

Poster/Beneficial Invertebrates - Monday, 16:30 - 17:30 BI-3-STU

Molecular dectection of *Noesma bombi* and *Crithidaia bombi* in wild and commercial populations of bumble bees in the U.S.

Anna Morkeski - University of Massachusetts - Amherst, Amherst, Massachusetts, USA; ***John Burand,*** Department of Plant, Soil and Insect Science, Amherst, Massachusetts, USA; ***Anne Averill,*** University of Massachusetts - Amherst, Amherst, Massachusetts, USA
Address for correspondence: jburand@microbio.umass.edu

Bumble bees are important pollinators in both agricultural and natural ecosystems. A variety of pathogens pose a threat to both wild and managed population of these bees. Polymerase chain reaction (PCR) based methods were developed for the detection of two microbial pathogens of bumble bees (*Bombus* spp.): *Nosema bombi* and *Crithidia bombi*. Specific and sensitive molecular detection techniques based on ribosomal sequences have previously been developed for the microsporidia, *Nosema bombi*, as well as *Nosema* infecting the honey bee (*Apis mellifera*). However,

ribosomal sequences of *N. bombi* can be difficult to analyze because they are present in high copy number and multiple sequence variants can be present in a single spore. Here, conserved regions of the RNA polymerase (RPB1) gene of *N. bombi* were identified and used to design primer sets to target these regions. To date, detection of the trypanosome, *C. bombi*, has been based entirely on observations made using light microscopy. Conserved regions of the beta-tubulin gene of related species were identified and primer sets were chosen from these regions to produce a 448 bp amplicon. Analyses of beta-tubulin and RPB1 sequences are being used to generate hypotheses about the prevalence and transmission of these parasites in U.S. *Bombus* populations.

Poster/Beneficial Invertebrates - Monday, 16:30 - 17:30 BI-4

Identification and characterization of a new microsporidium isolated from the silkworm, *Bombyx mori.*

Liangen Shi - College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, P.R. China
Address for correspondence: slgsilk@zju.edu.cn

The pebrine of the silkworm, Bombyx mori, is a disease caused by infection with the microsporidium Nosema bombycis. We have isolated a new microsporidian SLN1 from the 4th instar larvae in silkworm rearing farm of Zhjiang province, China. The mature spores of SLN1 were cylindrical or ovocylindrical in shape with a strong diopter and glossy surface. The spore size of SLN1 was 3.25±0.11µ2.05±0.12 um with a length/width ratio of 1.59±0.10 um, similar to those of Nosema bombycis. Therefore, the spores of SLN1 were hardly distinguished from the spores of Nosema bombycis under light microscope. The polar filament of the SLN1 spore with size of 59~75 um was significantly shorter than that of Nosema bombycis. In SLN1 spores formative stages, sporont produced pansporoblast including 8 nuclears by meiosis, and later 8 spores were formed in pansporoblast. SLN1 belongs to the genus Thelohania sp. The silkworm larvae were topically infected with SLN1. Infection was systemic with mature spores produced in epithelial cell of trachea, fat body, middle and posterior silkgland, fore and middle intestine, malpighian tubule and germ gland, most extensively, muscular tissue, but not in hind intestine, anterior potion of silkgland, dermal cells, nerve cells, and hemocyte cells. The IC_{50} value of SLN1 to newly-hatched silkworm larvae was 9200 spores/ml, 385-fold higher than that of Nosema bombycis, suggesting a weak infectiousness. SLN1 have transovarian transmissibility in silkworm, the rate of transovarian transmission was 3.51%, which was significantly lower than that of Nosema bombycis. The serological investigation using latex agglutination test with monoclonal antibodies against specific antigens on the surface of Nosema bombycis spores indicated that no cross-activation occurred, suggesting that it is totally different in serology between SLN1 and Nosema bombycis.

Poster/Beneficial Invertebrates - Monday, 16:30 - 17:30 BI-5

Presence and prevalence of viruses in local and migratory honeybees in Massachusetts.

Anna Welch - University of Massachusetts – Amherst, Amherst, Massachusetts, USA, ***John Burand*** - University of Massachusetts – Amherst, Amherst, Massachusetts, USA
Address for correspondence: jburand@microbio.umass.edu

In addition to their importance for honey production honeybees, *Apis mellifera*, play an important role in agriculture: they are pollinators of over 15 billion dollars of crop plants annually in the US. For example the over 600,000 acres of almonds grown in California requires approximately 1.2 million colonies of bees. The high demand for honeybee pollination services has resulted in a practice of commercial beekeepers moving large numbers of bees' around the country in order to meet this demand. Because of the nature of practices employed by many commercial beekeepers, migratory bees may experience conditions which render them more likely to become infected with pathogens. To begin to look at this possibility more closely we determined the presence and level of several viruses in migratory honeybees, and bees from hives maintained within a single location. Both the migratory bees and bees from local colonies were collect in late June and early July at approximately the same location in Eastern Massachusetts. Using multiplex RT-PCR, we analyzed the bees for five viruses, three of which were detected: Black queen cell virus (BQCV), Deformed wing virus (DWV), and Sacbrood virus (SBV). All three of these viruses were found in bees from both local and migratory hives, but at differing rates of infection. DWV was the most prevalent with 98% of the local bees being infected compared to 71.7% of the bees from migratory hives. BQCV was second most common virus found, with the trend reversed as 91.7% of the migratory bees were infected with BQCV while only 57.7% of the Massachusetts bees had this virus. The third virus, SBV, was detected in16% of the migratory bees and only 0.7% of the local bees.

FUNGI

Poster/Fungi - Monday, 16:30 - 17:30 F-1

withdrawn

Poster/Fungi - Monday, 16:30 - 17:30 F-2-STU

Gene expression analysis of *Beauveria bassiana* during infection of *Spodoptera exigua.*

Duriya Chantasingh - Mahidol University, Puthanomthon, Nakhon Pathom, Thailand; ***Kusol Pootanakit,*** Mahidol University, Puthanomthon, Nakhon Pathom, Thailand; ***Nemat Keyhani,*** University of Florida, Gainesville, Florida, USA; ***Lily Eurvilaichitr, Rungsit,*** National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand
Address for correspondence: mbkpn@mahidol.ac.th

The entomopathogenic fungus, *Beauveria bassiana*, with its broad host range, is an emerging model system with which to examine the molecular basis of fungal pathogenesis. Transcriptome analyses of *B. bassiana* derived from different cell types (aerial conidia, blastospore and submerge conidia) and culture conditions (chitin containing and oosporin producing media) has resulted in the identification of numerous candidate genes and their protein products implicated in pathogenesis. These genes include a suite of hydrolases that encompass proteases and chitinases, stress response genes such as superoxide dismutases, catalases, and peroxidases, and putative insect epicuticular hydrocarbon degrading enzymes. In order to probe the roles of some of these genes, their expression patterns were examined during infection of *Spodoptera exigua* using real-time PCR. In order to obtain kinetic data, expression analyses were performed using cDNA libraries generated from fungal-inoculated *S. exigua* larvae sampled every 2 hours from 0 hr to 14 hr. A dynamic pattern of gene expression was noted with several critical factors appearing to be constitutively present in the conidia. These data will help to identify genes which play important roles in the initial stages of infection. Moreover, this study provides a new perspective in the expression pattern of genes analyzed in during host infection.

Poster/Fungi - Monday, 16:30 - 17:30 F-3

Identification of a hybrid PKS-NPRS required for the biosynthesis of NG-391 and NG-393 metabolites in *Metarhizium anisopliae.*

Bruno Donzelli - USDA-ARS, Bio-IPM Research Unit, Ithaca, NY, United States; ***Stuart Krasnoff,*** USDA-ARS, Bio-IPM Research Unit, Ithaca, NY, United States; ***Alice Churchill,*** Dept. of Entomology, Ithaca, NY, United States; ***John Vandenberg,*** USDA-ARS, Bio-IPM Research Unit, Ithaca, NY, United States; ***Donna Gibson,*** USDA-ARS, Bio-IPM Research Unit, Ithaca, NY, United States
Address for correspondence: bdd1@cornell.edu

A 19,818 kb genomic region harboring six predicted ORFs was identified in *M. anisopliae* ARSEF 2575. The ORF4 CDS, putatively encoding a hybrid polyketide synthase/nonribosomal peptide synthetase (PKS-NRPS), was targeted using *Agrobacterium*-mediated gene knockout. Homologous, but not heterologous, recombinants failed to produce detectable levels of the fusarin-like compounds NG-391 and NG-393, indicating the involvement of this locus in their biosynthesis. ORF4 deletion mutants had no significant changes in virulence levels against *Spodoptera exigua* larvae and in resistance to H_2O_2 -generated oxidative stress compared to the wild type. The use of the ORF4 promoter-sGFP reporter fusion showed that the gene is transcriptionally regulated and expressed *in vitro* during early exponential growth; inoculum amount also affected expression, indicating a correlation with growth phase. RT-PCR performed using total RNA extracted during the fungal interaction within the *S. exigua* host revealed that ORF4 is expressed during the infection process.

Poster/Fungi - Monday, 16:30 - 17:30 F-4-STU

Effect of water activity and nitrogen source on *Isaria fumosorosea* propagule characteristics.

Francisco Escobar Herrera - CIAD A.C., Hermosillo, Sonora, Mexico, ***Veronica Mata,*** CIAD A.C., Hermosillo, Sonora, Mexico, ***Ali Asaff,*** CIAD A.C., Hermosillo, Sonora, Mexico
Address for correspondence: paco_colibri@hotmail.com

The entomopathogenic fungus, Isaria fumosorosea one of the most infective fungal species for the whitefly sp., can be cultured by submerged fermentation, growing as freely dispersed mycelia, mycelia aggregated into pellets, short hyphae, blastospores (B) and submerged conidia (SC). We studied the influence of water activity (a) and nitrogen source on the fungal growth pattern. The agar was modified employing glucose and/or polyethylene glycol (PEG MW 200, Sigma) at two levels (10 and 100 g/L and 0 and 100 mL/L respectively) and glutamate or NH_4NO_3 as nitrogen sources (at the same 20:1 C:N ratio) in two independent 22 factorial experimental designs, employing a mineral medium including per liter: 2 g KH_2PO_4, 2 g $MgSO_4$, 20 mg $FeSO_4.7H_2O$ and 20 mg ZnSO 4 .7H_2O, pH 5.5. 125 mL Erlenmeyer flasks, containing 30 mL of the inoculated culture media were incubated at 28ºC, at 100 rpm during 5 days. Pellets formation was measured as dry weight, in pre-weighed polyester organza screens (60 Mesh, 250 um). Blastospores and SC were counted in a hemacytometer using a light microscope (400µ magnification). Volume and morphological complexity of filtrate propagules were determined by flow cytometry (FACSCanto II, Becton Dickinson). Complexity and size were correlated to specific fungal structures by microscope observation. In all cases, glutamate containing media favored pellets formation; thus biomasses retained in the screens were around 100% higher than those including NH<4/sub>NO<3/sub>. Independently of nitrogen source, PEG diminished pellets formation and stimulated 6-10 times B and SC production; besides, flow cytometry showed that PEG and high glucose concentrations contributed to homogeneity of propagules population, showing that 85-95% of it had similar volume and complexity, comprising mainly SC. At low glucose concentration, and without PEG, more structures with a broad range of complexity and size were produced, which include unusual small B and SC (5-10%), normal B and C (50-70%), short and large hyphae (20-30%).

Poster/Fungi - Monday, 16:30 - 17:30 F-5

***Beauveria bassiana, Metarhizium anisopliae,* and *M. anisopliae* var. *acridum* conidia: Tolerance to imbibitional damage.**

Marcos Faria - Cornell University, Ithaca, NY, USA; ***Ann Hajek,*** Cornell University, Ithaca, NY, USA; ***Stephen Wraight*** - USDA-ARS, Ithaca, NY, USA
Address for correspondence: steve.wraight@ars.usda.gov

When dry fungal cells are immersed in water, rapid imbibition (water uptake) may compromise the plasma membrane, killing the cell. This study investigated the impact of imbibitional damage (measured in terms of reduced viability) on *Beauveria bassiana* (Bb), *Metarhizium anisopliae* (Ma) and *M. anisopliae* var. *acridum* (Mac) conidia dried/hydrated to five different water activities prior to immersion in water at temperatures varying from 0.5 to 33ºC. Imbibitional damage to conidia of all species occurred rapidly, with no differences in viability observed following immersion for 2 vs. 60 min. For Bb, initial water activity (aw) had little or no effect on germination following immersion at temperatures 15ºC. However, following immersion at 0.5ºC, dry conidia (aw = 0.335), moisture content (MC = 8.4%) retained only 43–65% viability compared to 87% for conidia that were partially or nearly fully hydrated (aw = 0.627; MC = 15.1%) prior to immersion. For Mac, immersion of dry conidia at temperatures ? 33ºC significantly reduced viability, but hydrated conidia (aw = 0.634 or 0.961; MC = 13.0%) were injured only at 0.5ºC. Dry Ma conidia with aw in the 0.026-0.333 range (2.1–8.4% MC) showed reduced viability after immersion at all temperatures, and viability dropped to 1% and 27–41% following immersion in water chilled to 0.5 and 15ºC, respectively. Immersion of dry Ma conidia at 25 and 33ºC resulted in viabilities of 66–75% and 86%, respectively. Viabilities observed following immersion of partially hydrated Ma conidia (0.632 aw; MC = 14.8%) were greater than those observed after immersion of dry conidia at temperatures < 33ºC, but highest viabilities (91-94%) were observed after immersion of fully hydrated conidia (0.957 aw; MC = 39.7%) regardless of temperature. Suspension of dry Ma conidia in paraffinic oil conferred protection against imbibitional damage.

Poster/Fungi - Monday, 16:30 - 17:30 F-6

Biology of Mormon Cricket *Anabrus simplex* and laboratorycolony development.

Rodrigo B. Ferreira - Utah State University, Logan, UT, USA; ***Éverton K. K. Fernandes,*** Utah State University, Logan, UT, USA; ***Chad A. Keyser,*** Utah State University, Logan, UT, USA; ***Scott Treat,*** Utah State University, Logan, UT, USA; ***Donald W. Roberts,*** Utah State University, Logan, UT, USA
Address for correspondence: dwroberts@biology.usu.edu

The Mormon cricket (MC), an important agricultural pest of the Western United States, is the focus of a project to develop fungal biocontrol agents for use against MC outbreaks in areas unsuitable for chemical pesticides. Insect-pathology research on MC currently relies on field-collected specimens, with unknown disease and parasite loads, age, nutritional history, etc. Since, this species is univoltine, specimens are available less

than five months per year. Accordingly, the present study attempts to establish a laboratory colony. MC eggs were collected in northeast Utah, during November, 2008. The eggs were held in plastic screen-topped centrifuge tubes (50ml), incubated at 11, 15, or 20ºC, sprayed with water each day, and checked daily for hatch. Optimum temperature for hatch was 15ºC (N= 33; 82.5%), with the average time to hatch 15 days. MC nymphs were individually maintained in cages with autoclaved sand, polyacrylamide water gel, commercial cricket food mix, sunflower seeds, tropical fish flakes, and organic lettuce. Gender identification was possible by day 11. On average, males and females achieved adulthood in 38-40 days. Adult male length was 39.7 to 49.4 mm, and female adults 42.5 to 51.6 mm. Male adult weights ranged from 3.08 to 5.94 g, and females from 3.65 to 7.8 g. Copulation occurred with 40% of couples held in individual cages. Of the couples not copulating, 84% had at least one individual with a physical disability. A positive correlation was observed between male weight-loss and female weight-increase after copulation. A positive correlation was observed between MC weight and participation in copulation, viz. MC that copulated tended to be heavier. The preferred time of day for egg laying was late afternoon. The number of eggs laid each day after copulation varied from 1 to 138, average total eggs per female was 349. Mormon crickets lived 79 days on average, and males generally survived longer than females. Future research will emphasize ideal conditions for embryo development.

Poster/Fungi - Monday, 16:30 - 17:30 F-7

Genotypic diversity of *Beauveria bassiana* isolates in acridids from the Northern Plains of the United States.

Stefan Jaronski - USDA-ARS, Sidney, MT, USA, ***Paul Kaufmann,*** USDA-ARS, Sidney, MT, USA; ***John Gaskin,*** USDA-ARS, Sidney, MT, USA
Address for correspondence: stefan.jaronski@ars.usda.gov

Beauveria bassiana is naturally present in grasshopper populations of the U.S. Northern Plains. It is often rare in a population, but at times can reach a prevalence of 10-15%. One strain, GHA, is registered in the U.S. for use against grasshoppers as well as other insects. We explored the genotypic variation among 60 *B. bassiana* isolates obtained in 2000 from different grasshopper hosts in 6 separate geographic locations in North Dakota and Montana, North America. All isolations were made from insects alive at time of collection, but subsequently succumbing to infection. Three non-U.S. isolates (ARSEF4197 from Russia, ARSEF357 from Australia and ARSEF1053 from Brazil) were included for comparison; ARSEF 1053 was used as an out-group in some of our analyses. For analysis we employed the ITS1 and ITS2 region nuclear and ribosomal DNA sequences, using ITS1F and ITS4 primers, and AFLP with EcoR1 and Mse1 restriction enzymes and EcoR1-ACC and EcoR1-AGC extensions. ITS sequencing produced five groups, distinguished by the number of changes in the ITS sequence based on mutations at 20 loci. No ITS groups had any correlation with collection site or acridid host species. The largest group, of 41 isolates, included the nonindigenous ARSEF357 and 4197. The other groups had 11, 6 and 2 isolates respectively, while the Brazilian ARSEF1053 occupied its own group. AFLP yielded 207 polymorphic bands. Genotypic distances among every isolate within any particular ITS group ranged between 0.29 and 0.87. Future studies extending our observations to additional isolates from grasshoppers, and from the soil from the collection sites are planned.

Poster/Fungi - Monday, 16:30 - 17:30 F-8

Genome profiling in hybrid strains of entomopathogenic fungi *Lecanicillium* spp. *(Verticillium lecanii).*

Masanori Koike - Obihiro University, Obihiro, Hokkaido, Japan; ***Fusako Kaibara,*** - Kyushu University; ***Daigo Aiuchi,*** - Obihiro University; ***Yoshinori Hatakeyama,*** - College of Bioresource Sciences, Nihon University; ***HIdetoshi Iwano,*** - College of Bioresource Sciences, Nihon University
Address for correspondence: koike@obihiro.ac.jp

The entomopathogenic fungi *Lecanicillium* spp. which have wide host range and high virulence toward various arthropods are exploited commercially as useful biological control agents all over the world. Vertalec (*L. longisporum*) and Mycotal (*L. muscarium*) have high specific virulence against aphids and whiteflies, respectively. B-2(*L. muscarium*), which was isolated in Japan, has a high ability to colonize on cucumber leaves. These three strains were fused to each other to obtain hybrid strains that have wide host specificity and persistence of an effect in low humidity(Aiuchi et al., 2008). We investigated diagnostic DNA fragments among these parental strains and some hybrid strains in order to detect molecular markers to distinguish those of strains. Unique banding patterns generated from genome profiling (GP) for comparing amount of information derived from a genetic sequence could be detected among hybrid strains. A dendrogram created by unweighted pair group method analysis (UPGMA) of GP date reflected previous results. These findings suggest that GP may be effective method for discriminating closely-related strains in these cases.

Poster/Fungi - Monday, 16:30 - 17:30 F-9

A yeast-like symbiont of *Tenebrio molitor* and *Zophobas atratus.*

Jeff Lord - USDA-ARS, Manhattan, KS, USA
Address for correspondence: jeff.lord@ars.usda.gov

An investigation into the cause of nation-wide collapses in commercial *Tenebrio molitor* cultures led to the discovery of a unicellular symbiont that infects the fat body and ventral nerve chord of adult and larval beetles. In adult males, there is heavy infection of the epithelial cells of the testes and between testes lobes with occasional penetration of the lobes. In adult females, spores are associated the spermatheca but have not been found in the ovaries. This suggests that there is venereal transmission and per os transmission via externally contaminated eggs. Pathogenicity, if it exists, is chronic. The identity of the symbiont is to be revealed in this presentation.

Poster/Fungi - Monday, 16:30 - 17:30 F-10

Immune response of Mormon crickets to infection by *Beauveria bassiana.*

Robert Srygley - USDA-Agriculture Research Service, Sidney, MT, United States; ***Stefan Jaronski,*** USDA-Agriculture Research Service Sidney, MT, United States
Address for correspondence: robert.srygley@ars.usda.gov

The Mormon cricket (*Anabrus simplex*), a tettigoniid, is a major pest of crops and rangeland in the western United States. *Beauveria bassiana* is an entomopathogenic fungi that serves as a biological control agent of this pest and other grasshoppers. Adult Mormon crickets were drawn from a topical bioassay of *B. bassiana* Strain GHA using doses of $5.13x10^4$-$1.75x10^6$ conidia in sunflower oil, with oil only as a control. After incubating the insects for three weeks at 28°C, by which point mortality ranged from 25-80%, we assessed hemolymph phenoloxidase (PO) and lysozyme-like activities of survivors (five males and five females for each treatment, fewer if there were not enough survivors), and scanned a sample of their hemolymph for fungal cells. As expected,

adult mortality increased with conidial dose, and there was a significant decrease in body mass that generally paralleled the dose. Phenoloxidase activity was significantly different between treatment groups, but only the controls had significantly less PO activity in a post hoc comparison of the means. There was no difference in lysozyme-like activity between the treatments. Hence Mormon crickets had elevated levels of PO but not lysozyme in defense against *Beauveria* infection. PO activity was elevated to the same level independent of infection intensity (dose) in these surviving insects. PO activity of survivors with fungal cells visible in their hemolymph did not differ significantly from those with clear hemolymph. We conclude that circulating PO may be an important enzymatic defense against *Beauveria* infection, and that it is associated with attempted clearing of *Beauveria* blastospores and hyphae from the hemolymph of Mormon crickets.

Poster/Fungi - Monday, 16:30 - 17:30 F-11

Statistical considerations in the analysis of data from replicated bioassays.

Stephen Wraight - USDA-ARS, Ithaca, New York; USA; ***Stefan Jaronski,*** USDA-ARS, Sidney, Montana, USA; ***Mark Ramos,*** USDA-ARS, Ithaca, New York, USA; ***Michael Griggs,*** USDA-ARS, Ithaca, New York, USA; ***John Vandenberg,*** USDA-ARS, Ithaca, New York, USA
Address for correspondence: steve.wraight@ars.usda.gov

Multiple-dose bioassay is generally the preferred method for characterizing virulence of insect pathogens. Linear regression of probit mortality on log dose enables estimation of LD50/LC50 and slope, the latter having substantial effect on LD90/95s (doses of considerable interest in development of pathogens for pest management). Susceptibility of arthropods to pathogenic microorganisms varies markedly with time, resulting in high between-assay variability. This can become problematic, as assays are more time and resource intensive than single-dose tests, and experiments involving more than a few treatments may necessitate repetition on different occasions using different batches (usually different generations) of test insects. In addition, because of the inherently high variability of assays, demonstration that results are repeatable over time is desirable and often demanded by journal editors and reviewers. Our findings are from replicated bioassays of commercially-produced conidia of the entomopathogenic fungus *Beauveria bassiana* strain GHA against larval hemipteran, lepidopteran, coleopteran, and orthopteran hosts and include observations on the between-assay distributions and variability of commonly used bioassay statistics. Results indicate that replicated estimates of lethal doses, slopes, and lethal dose ratios from probit/logit regression analyses of repeated bioassays are amenable to traditional statistical analyses, including parametric ANOVA, an approach rarely discussed in general treatises on analysis of bioassay data, which tend to focus almost exclusively on within-assay variability (assay precision). Application of conventional ANOVA in analyzing statistics from probit/logit analyses provides unbiased estimates of mean lethal doses and slopes with realistic standard errors reflecting the full variability of host-pathogen interactions and offers numerous options for rigorous multiple comparisons. Greater application of this approach is recommended not only for its familiarity and rigor but also to provide more meaningful and reliable assessments of pathogen virulence.

Poster/Fungi - Monday, 16:30 - 17:30 F-12

Overexpression of manganese superoxide dismutase from *Beauveria bassiana* enhances its tolerance to oxidative stress.

Xue-qin Xie - Institute of Microbiology, Zhejiang University, Hangzhou, Zhejiang, P.R.China; ***Sheng-hua Ying,*** - Institute of Microbiology, Zhejiang University, Hangzhou, Zhejiang, P.R. China; ***Ming-guang Feng,*** - Institute of Microbiology, Zhejiang University, Hangzhou, Zhejiang, P.R. China
Address for correspondence: cherryxie36@163.com

Superoxide dismutases (SOD) are a ubiquitous class of metalloenzymes that play a crucial role in protecting organisms against toxic effects caused by ROS. In this study, the *Mn SOD* genes, designated *BbSod2,* were cloned and characterized from the important fungal biocontrol agent,*Beauveria bassiana* (Bb). *BbSod2* gene (802bp) corresponded to a 209 amino acid polypeptide with a predicted molecular mass of 23.2 kDa. A database search for sequence homology revealed the deduced amino acid sequence shows highest identity rate with *Mn-SOD* from *Neurospora crassa* (81%). All conserved amino acids of the *Mn-SOD* family, including the Parker-Blake signature and four metal-binding residues, are present in the predicted *BbSod2* protein. The purified recombinant enzyme was stable at pH6-10 and temperatures up to 40°C. Overexpression of *BbSod2* in Bb strain devoid of this enzyme enhances its tolerance to the oxidative agent.

Poster/Fungi - Monday, 16:30 - 17:30 F-13

Thioredoxin from the entomopathogenic fungi, *Beauveria bassiana*: Gene cloning, characterization and functional expression.

Sheng-hua Ying - Institute of Microbiology, Zhejiang University, Hangzhou, Zhejiang, P.R. China; ***Xiao-hui Wang,*** - Institute of Microbiology, Zhejiang University, Hangzhou, Zhejiang, P.R.China; ***Ming-guang Feng,*** - Institute of Microbiology, Zhejiang University, Hangzhou, Zhejiang, P.R. China
Address for correspondence: yingsh@zju.edu.cn

An EST of *Beauveria bassiana* was reported to have identity to the thioredoxin. The cloning, identification and functional expression of the first thioredoxin in entomopathogenic fungi were carried out in this study. The *B. bassiana* thioredoxin has not only similarity to other characterized thioredoxin in conservation of redox active site but also an additional C-terminus. The recombinant thioredoxin (BbTrx) and its domain (BbTrxD) were expressed and purified into homogeneity. By comparison of the concentration dependency and thermal stability of BbTrx and BbTrxD, the *B. bassiana* thioredoxin was proved a typical member of thioredoxin family. The C-terminus of the BbTrx might also contribute to the binding affinity to the target protein and influence on the protein structure under stress temperature, but not necessary for the catalytic activity. The Logistic model was successfully used to describe the catalytic characteristics of thioredoxin and its parameters reflected the biochemical traits of the thioredoxin. Because that overexpression of BbTrx in *E. coli* could confer the acquired thermal stability to the host cells, the BbTrx is a potential candidate thioredoxin gene for construction of engineered fungi or other organisms with improved environmental fitness.

Poster/Fungi - Monday, 16:30 - 17:30 F-14

Study of temperature-growth interactions of entomopathogenic fungi isolated from chalk grassland in the UK.

Emma Turner - Centre for Ecology and Hydrology, Oxford, Oxfordshire, United Kingdom; ***Helen Hesketh*** - Centre for Ecology and Hydrology, Oxford, Oxfordshire, United Kingdom; ***Rosie Hails, Oxford,*** - Centre for Ecology and Hydrology, Oxfordshire, United Kingdom

Address for correspondence: hhesketh@ceh.ac.uk

The majority of studies on insect population regulation by entomopathogenic fungi have been in the context of pest control. There has been little research into how fungi may impact on natural populations of insects. The Adonis Blue (Polyommatus bellargus) and Chalkhill Blue (Polyommatus coridon) butterflies are species of Lepidoptera with particular conservation interest in the UK. Both of these species exist on the most northern edge of their climatic range in populations that are restricted to fragmented chalk grassland habitats in the South of England. Larvae of both butterflies feed on the same single host plant species (*Hippocrepis comosa*) and can be found in the soil during their development. It is therefore reasonable to assume that larvae will be exposed to soil entomopathogenic fungi. *Polyommatus bellargus* are bivoltine, and the distribution of early season larvae is very closely linked to warmer temperatures in sheltered microhabitats. The use of turf height and shelter category can accurately predict the occupancy of sites across a landscape for *P. bellargus* and define optimal and sub-optimal habitats, and it is thought a similar relationship exists for *P. coridon*. A combination of host- and habitat- selection could result in local adaptation of entomopathogenic fungi. Because shelter category is a proxy for temperature, it is possible that one of the driving selectors is the ability of fungi to operate at certain temperatures. Soil was collected from these habitats and turf height and shelter category was recorded. A total of 130 entomopathogenic fungi were extracted from 144 soil samples using *Galleria melonella* as bait. The in vitro growth of 40 of these isolates was investigated at a range of four different temperatures between 10 and 25ºC. The in vitro growth of a number of selected isolates was then profiled at a wider range of temperatures.

MICROSPORIDIA

Poster/Microsporidia . Monday, 16:30 - 17:30 M-1

Predation by the carabid beetle, *Calosoma sycophanta* affects transmission of microsporidia infecting gypsy moth larvae.

Dörte Goertz - University of Natural Resources and Applied Life Sciences, Vienna, Austria; ***Gernot Hoch,*** - Resources and Applied Life Sciences, Vienna, Austria
Address for correspondence: doerte.goertz@boku.ac.at

In a series of projects, we have been studying the horizontal transmission of microsporidia that infect the gypsy moth, *Lymantria dispar*. *Vairimorpha disparis* and *Nosema lymantriae* differ in their life cycle, virulence, host tissues that are infected, and transmission pathways. The interactions between a host and its pathogens can be influenced by several factors such as other pathogens, parasitoids or predators. In this poster, we report on the impact of the predatory carabid beetle, *Calosoma sycophanta*, on transmission of *N. lymantriae* and *V. disparis*. The beetles disseminated spores of both pathogens with their feces and during feeding on microsporidia-infected larvae. This resulted in a higher percent infection in *L. dispar* test larvae with N. lymantriae and V. disparis when exposed on such contaminated foliage. First results indicate that C. sycophanta beetles preferred infected over uninfected larvae when given the choice. An experiment on caged, potted oak plants showed that the predator affected the transmission success of N. lymantriae and V. disparis differently. When C. sycophanta was allowed to prey on infected larvae at an early stage of infection, the transmission success of V. disparis increased while the transmission success of N. lymantriae was not affected. The difference is likely due to the different routes of horizontal transmission: N. lymantriae spores are already released from living larvae with feces, while spore release of V. lymantriae usually begins after death of the host. Predation by C. sycophanta apparently led to an earlier dissemination of spores. When C. sycophanta was allowed to prey on infected larvae later, the transmission success of both pathogen species was reduced. C. sycophanta never became infected with N. lymantriae or with V. disparis after feeding on infected prey.

Poster/Microsporidia . Monday, 16:30 - 17:30 M-2

Temperature and simulated rain affect horizontal transmission of the microsporidium *Nosema lymantriae.*

Gernot Hoch - University of Natural Resources and Applied Life Sciences, Vienna, Austria; ***Sieglinde Pollan,*** - Resources and Applied Life Sciences, Vienna, Austria; ***Carina Steyer,*** - Resources and Applied Life Sciences, Vienna, Austria; ***Dörte Goertz,*** - Resources and Applied Life Sciences, ***V***ienna, Austria
Address for correspondence: doerte.goertz@boku.ac.at

Nosema lymantriae uses two main routes for horizontal transmission among Lymantria dispar host larvae. Spores are released from living larvae with feces and from cadavers after host death. The time lag between infection of a host and the onset of its infectious phase is important for the success of horizontal transmission. When infected *L. dispar* larvae were reared at different temperatures (18°C, 21°C, 24°C) in the laboratory, significant effects on disease progress as well as spore release were ascertained. Developmental stages and spores of *N. lymantriae* occurred earlier in silk glands, fat body and Malpighian tubules of infected larvae when larvae were reared at higher temperatures. Spores were released with feces seven days earlier at 24°C (13.3±0.2 days post inoculation) than at 18°C, and two days earlier than at 21°C. Moreover, the mean amount of spores released differed significantly: 2.7x10 7 ± 7.0x10 6 spores were released at 24°C while 3.1x10 6 ± 1.5x10 6 were released at 18°C. We conclude that higher temperatures within the tested ranges will lead to a faster increase of the amount of inoculum in the environment of *L. dispar* larvae and consequently increase the likelihood of new infections in the population. An experiment utilizing oak twigs in 9.1-liter containers in which we excluded infected larvae but only allowed dropping feces to contaminate leaves proved that spores originating from oak leaves as food play a role in horizontal transmission. Of test larvae in these containers, 4.4±2.4 % acquired infections. When we simulated rain of light intensity with a watering can, horizontal transmission increased significantly and 30.0±8.2 % of test larvae became infected. Light rain may enhance transmission also in the field by increasing the number of fecal pellets that stay on the foliage as well as contamination leaf surfaces with spores.

Poster/Microsporidia - Monday, 16:30 - 17:30 M-3

High temperature eliminates microsporidia from an insect host.

Johny Shajahan - Illinois State University, Sault Ste. Marie, Ontario, Canada; ***Austin Omer*** - Illinois State University, Normal, Illinois, USA; ***William Newgent*** - Illinois State University, Normal, Illinois, USA; ***Katriona Elmer -*** Illinois State University, Normal, Illinois, USA; ***Douglas W. Whitman*** - Illinois State University, Normal, Illinois, USA; ***Regina Stoerger,*** - Dept. of Biology, Illinois State University, ***Will Hatch,*** - Dept. of Biology, Illinois State University
Address for correspondence: johny.shajahan@nrcan.gc.ca

Some hosts have higher temperature tolerances than their pathogens, and thus can use high temperature to fight pathogenic infections. For example, many insects solar-bask when under pathogen attack, and such “behavioral fever” is thought to harm pathogens and aid insect survival. We tested the ability of heat to reduce levels of the pathogenic

microsporidium, *Encephalitozoon romaleae,* infecting the grasshopper *Romalea microptera.* We subjected grasshoppers to 10 d of a repeating thermoperiod of 14 h of high temperature (up to 46°C), interspersed with 10 h of medium temperature (up to 37.5°C), combined with a daily 10-min acute heat shock (up to 52.5°C). Our results show a 99% reduction in spore count in heat-treated insects as compared to control animals. In 56% of the treated grasshoppers, we were unable to find a single spore, suggesting that our heat treatment had eliminated the pathogen. In a second experiment, we tested the effects of acute heat shock alone. We maintained grasshoppers for 10 days under normal rearing temperatures of 32°C (day) and 28°C (night), but subjected the animals to10-min heat shocks (of up to 56°C) on Days 1, 4, and 7 (i.e., three heat shocks, total, during 10 d). We observed a significant reduction (70%) in spore count, compared to control animals; however, acute heat shock, alone, was not as effective at eliminating microsporidia spores as was the combination of chronic + acute high temperatures (Experiment 1). Our data imply that perhaps long periods of moderately high temperatures may be more effective at reducing pathogens than short periods of extremely high temperatures (acute heat shocks). Overall, our results support the hypothesis that cold-blooded animals can combat disease by behaviorally increasing their body temperatures. Our results also suggest that researchers can reduce or eliminate pathogens in laboratory colonies of insects by applying heat treatments.

Poster/Microsporidia - Monday, 16:30 - 17:30 M-4-STU

Characterization of a microsporidia isolated from S. *litura* and its relationship with a nucleopolyhedrovirus in Vietnam.

Thao Le Thi Thanh - Student, Tokyo, Japan; ***Madoka Nakai*** - Associate Professor, Tokyo, Japan; ***Yoshinori Hatakeyama*** - Research Assistant, Kanagawa, Japan; ***Iwano Hidetoshi*** - Professor, Kanagawa, Japan; ***Yasuhisa Kunimi*** - Professor, Tokyo, Japan
Address for correspondence: 50007539718@st.tuat.ac.jp

A microsporidia isolated from diseased larvae of *Spodoptera litura* collected in the soybean field in Cantho, Vietnam was characterized. Phylogenetic analysis based on the 16S small subunit rRNA gene sequences shows that this isolate is closely related to *Nosema bombycis* . Mature spores were produced in the midgut, tracheae, Malpighian tubules and fat body tissues. The LD^{50} values for neonatal and fourth instar larvae of *S. litura* were determined as 8.9 x 10< sub>2 < sub>and 1.4 x 10< sub>6< sub> spores per insect, respectively. The effect of combinations of several lethal doses of *N. bombycis* and *S. litura* nucleopolyhedrovirus on second instar larvae of *S. litura* was examined. Mortality data from larvae exposed to both pathogens simultaneously indicated that the effects of the pathogens in combination were mostly independent but antagonistic effects occurred at the high dose of NPV.

Poster/Microsporidia - Monday, 16:30 - 17:30 M-5

New microsporidia-host association: four new species infecting bark lice (Psocoptera).

Yuliya (Julia) Sokolova - Institute of Cytology Russian Academy of Sciences, St.Petersburg, Russia; ***Igor Sokolov*** - Louisiana State University AgCenter Entomology Dept., Baton Rouge, LA, USA; ***Christopher Carlton*** - Louisiana State University AgCenter Entomology Dept., Baton Rouge, LA, USA
Address for correspondence: jumicro@yahoo.com

Terrestrial microsporidia have been reported mainly from species of economic importance, but the prevalence and role of microsporidia in non-agricultural terrestrial ecosystems are practically unknown. This lack of data impedes progress in phylogenetic studies and development of natural systems of classification for microsporidia. Insects captured in Malaise canopy traps in Great Smoky Mountains National Park were examined for evidence of microsporidian infections. Captured insects were fixed in polyethylene glycol. Two Psocoptera species, *Xantocaecilius sommermanae* and *Polypsocus corrupotus*, displayed symptoms of microsporidiosis. Presence of microsporidia was confirmed by light and electron microscopy and by PCR amplification. The material had been stored in glycol for three summer months but, unexpectedly, displayed fair DNA preservation and was partly suitable for electron microscopy. Psocopterans were infected with four microsporidia species that are new to science. SSU-rDNA-based phylogenetic analysis suggests that two new species cluster with a group of microsporidia from terrestrial insects, related to the *Paranosema-Antonospora* clade, while the other two group with the *Encephalitozoon-Nosema* clade. The discovery of microsporidia belonging to distantly related lineages in bark lice suggests that these ancient insects or their ancestors might have played an important evolutionary role in establishing the host range of contemporary microsporidia. Psocoptera belong to a group of orders that includes Hemiptera (true bugs, plant hoppers, aphids, etc.). Further, Psocoptera are believed to be the sister taxon to Phthiraptera, or lice, that parasitize warm blooded animals, including humans. Documenting phylogenetic relationships of discovered species and further surveys of microsporidia in Psocoptera and related orders may reveal avenues of host transfer across related and unrelated lineages. The finding that samples stored for months in glycol coolant are suitable for morphological and DNA analysis is surprising and important. This discovery opens new horizons in microsporidia research. Supported by Discover Life in America, Small Grant Program 2008.

VIRUS

Poster/Virus . Monday, 16:30 - 17:30 V-1-STU

Cloning and recombinant expression of glycoprotein e2 of the classical Swine Fever virus.

Sung-Min Bae - Chungbuk National University, Cheongju, Chungbuk, Korea; ***Hyun-Na Koo*** - Chungbuk National University, ***Yeon-Ho Je,*** - Seoul National University; ***Byung-Rae Jin,*** - Dong-A University; ***Soo-Dong Woo*** - Chungbuk National University
Address for correspondence: sdwoo@cbnu.ac.kr

The Classical Swine Fever Virus (CSFV) is a member of the Pestivirus genus of the Flaviviridae. The genome of CSFV is a positive singlestranded RNA molecule 12.3 kb and contains a single large open reading frame (ORF). The polyprotein composed of eight nonstructural and four structural proteins (nucleocapsid protein C and three envelope glycoprotein E0, E1 and E2). E2, the most immunogenic of the CSFV glycoproteins, induces a protective immune response in swine. To determine the characteristics of the CSFV, LOM strain, we investigated the nucleotide sequence of the glycoprotein E0, E1 and E2. Comparison of the LOM with the other strains revealed nucleotide sequence identity ranging from 97 to 98%. Expression of the glycoprotein E2 was identified by SDS-PAGE and Western blot analysis using anti-CSFV E2 monoclonal antibodies in Sf21 cells. The expression levels of glycoprotein E2 were observed from day 3 and 5 days maximum. In addition, its expression efficiency by media and cell line was investigated. The result showed that High-Five cells and Grace's insect media for Sf21 were the best conditions for the expression of the glycoprotein E2.

Poster/Virus - Monday, 16:30 - 17:30 V-2

Further studies on divalent cations and transmission of CUNIDVB in *Culex quinquefasciatus.*

James Becnel - USDA/ARS/CMAVE, Gainesville, Florida, USA
Address for correspondence: james.becnel@ars.usda.gov

CuniDBV is a member of the Dipteran–specific baculoviruses in the genus Deltabaculovirus (DBV) that specifically infects mosquito larvae within the genus *Culex*. Infections are restricted to the nuclei of larval midgut epithelial cells where occlusion bodies (OB's) are produced. Mature OBs of CuniDBV are uniform, globular shaped particles with a diameter of approximately 400 nm and are infectious per os to mosquito larvae. High transmission levels are dependent upon divalent cation concentrations and ratios. Exposure of third instar larvae to virus ($1e^8$ OB's/ml) together with magnesium at a concentration of 10 mM produces 80-100% infection levels at 48 hours post-exposure. Virus exposures in the presence of calcium at 10 mM do not result in infected larvae at 48 hours post-exposure. When both magnesium and calcium are present, infection of larvae is greatly reduced at ratios as low as 5:1 (magnesium to calcium). In addition, other divalent cations can function as activators and inhibitors of CuniDBV transmission. Activators include barium, cobalt, nickel and strontium, while additional inhibitors of transmission are copper, iron and zinc. While relatively high magnesium concentrations are required for infection, nickel is a particularly potent activator at concentrations as low as 0.2 mM. Forty-eight hour infection levels have proven to be convenient for laboratory bioassays but it may not be reflective of the actual infection levels in exposed larvae particularly at lower divalent cation concentrations. In addition, combinations of activators have not been evaluated on CuniDBV infections. In this study, we examine the effects of various concentrations of magnesium and nickel (alone and in combination) on the time course of CuniDBV infections in larvae beyond 48 hours post-exposure. Results will be used to develop formulations that combine divalent cations and CuniDBV for field applications.

Poster/Virus - Monday, 16:30 - 17:30 V-3

Proviral structure and organization of *Cotesia congregata* bracovirus.

Annie Bezier - IRBI CNRS Université de Tours, Tours, Region Centre, France; ***Gabor Gyapay*** - CEA Genoscope, Evry, Ile de France, France; ***Georges Periquet*** - IRBI CNRS Université de Tours, Tours, Region Centre, France; ***Elisabeth Herniou*** - IRBI CNRS Université de Tours, Tours, Region Centre, France; ***Jean-Michel Drezen*** - IRBI CNRS Université de Tours, Tours, Region Centre, France
Address for correspondence: annie.bezier@univ-tour.fr

Polydnavirus (PDV) are double-stranded DNA viruses associated with tens of thousands species of parasitic wasps that develop within the body of lepidopteran larvae. Their genomes exist in two distinct forms. The proviral genome is permanently integrated into the parasitic wasp genome and vertically transmitted to the offspring. The packaged genome produced from the proviral form is injected along with parasite eggs into the host body to deliver genes encoding virulence factors used to manipulate the physiology of the parasitized host. Here we present the analysis of Cotesia congregata bracovirus (CcBV) proviral genome. We show that segments are generally clustered but located at several positions in the wasp genome, separated by regions containing wasp genes and mobile elements. However a major locus (700 kilobases) comprises 65 % of the segments. The genes encoded by clustered segments often contain genes belonging to the same gene families, suggesting they have been produced by segmental duplications. Most duplicated segments are found in both Cotesia congregata and the recently sequenced Glyptapanteles proviral forms of bracoviruses. Moreover comparisons of proviral forms reveal conserved features between Cotesia and Glyptapanteles bracovirus genomes consistent with their inheritance from a common wasp ancestor.

Poster/Virus - Monday, 16:30 - 17:30 V-4

withdrawn

Poster/Virus . Monday, 16:30 - 17:30 V-5

Systemic pathogenesis of ACMNPV budded virus in *Anticarsia gemmatalis* larvae.

Eric Haas-Stapleton - California State University, Long Beach, Long Beach, CA, USA; ***Alisa De La Cruz*** - California State University, Long Beach, Long Beach, CA, USA; ***Elisa Martinez*** - California State University, Long Beach, Long Beach, CA, USA; ***Marianne Torres,*** - California State University, Long Beach, Long Beach, CA, USA; ***Tiffany Chen*** - California State University, Long Beach Long Beach, CA, USA
Address for correspondence: ehaas@csulb.edu

We have investigated infection and pathogenesis of *Autographa californica* M Nucleopolyhedrovirus in penultimate instar *Anticarsia gemmatalis*, using a lacZ recombinant virus (AcMNPV-*hsp70/lacZ*) to track the temporal progression of infection in the hemocoel. *A. gemmatalis* was highly resistant to fatal infection by budded virus (BV) administered via the systemic route (LD50>3 x 10^5 pfu). Time-course studies showed BV did not efficiently disseminate infection and only cuticular epidermal cells displayed high levels of viral infection. Flow cytometry analysis of hemocytes isolated from BV?inoculated *A. gemmatalis* larvae showed low level expression of the BV envelope protein GP64 on the cell surface, pointing to a limited capacity for A. gemmatalis hemocytes to amplify virus in the insect. However, hemocytes infected *ex vivo* with AcMNPV*hsp70/ lacZ* expressed the lacZ reporter gene, suggesting that BV can enter and uncoat in hemocytes. In aggregate, our studies show that systemic AcMNPV infection in *A. gemmatalis* is limited by inefficient BV amplification.

Poster/Virus - Monday, 16:30 - 17:30 V-6

Sequence and analysis of the genome of the Illinois isolate of *Agrotis ipsilon* multiple nucleopolyhedrovirus.

Robert Harrison - Invasive Insect Biocontrol and Behavior Laboratory, USDA Agricultural Research Service, Beltsville, Maryland, USA
Address for correspondence: robert.l.harrison@ars.usda.gov

The Agrotis ipsilon multiple nucleopolyhedrovirus (AgipMNPV) is a group II nucleopolyhedrovirus (NPV) that is being tested for season-long, multi-year biological control of the black cutworm (Agrotis ipsilon) on golf courses. Genomic DNA from a larval polyhedra stock of the Illinois strain of AgipMNPV was isolated and completely sequenced. The AgipMNPV genome is 155,122 nt in size and contains 163 open reading frames (ORFs), including 61 ORFs found among all lepidopteran baculoviruses sequenced to date. Comparisons to other NPV genomes indicated that AgipMNPV is part of a discrete, well-supported clade of group II NPVs that includes viruses from Agrotis and Spodoptera host species. NPVs in this clade contain homologous repeat regions with the conserved core repeat sequence TTTGCTTT(N18–21)AAAGCAAA. Among NPVs that have been sequenced, AgipMNPV is most closely related to Agrotis segetum NPV-A (AgseNPV-A). The two viruses nevertheless have diverged significantly, with an average ORF amino

acid sequence identity of 70.6% (+/- 15.9%) and several differences in ORF content. A genotype characterized by a 128-bp deletion in the ecdysteroid UDP glucosyltransferase (egt) ORF was identified in contigs from the larval AgipMNPV sequence. Approximately half of individual AgipMNPV clones plaque-purified on the A. ipsilon cell lines AiE1611T and AiEd6T also contained this deletion.

Poster/Virus - Monday, 16:30 - 17:30 V-7

In vivo pathogenesis of *Lymantria dispar* M Nucleopolyhedrovirus using a VP39-GFP/HSP70/LACZ recombinant.

Kelli Hoover - Penn State University, University Park, PA, USA; ***James Slavicek*** - U.S. Forest Service, Delaware, OH, USA; ***Nancy Hayes-Plazolles*** - U.S. Forest Service, Delaware, OH, USA; ***James McNeil*** - Penn State University, University Park, PA, USA
Address for correspondence: kxh25@psu.edu

Pathogenesis of *Lymantria dispar* M nucleopolyhedrovirus (LdMNPV) was examined in fourth instar *L. dispar* during a time course experiment using a recombinant containing two reporter genes: the fusion protein vp39-GFP driven by a duplicated vp39 promoter and lacZ driven by hsp70. The purpose of this study was to determine if these reporters could be used to visualize and follow key steps in the progression of infection in the host, including binding and fusion of occlusion derived virus (ODV) to the midgut epithelial cells and subsequent replication in these cells. Our ultimate goal is to use these reporter genes to study single and double deletion mutants of the enhancin genes of LdMNPV to determine if one or both of the enhancins facilitate binding and/or fusion of ODV to midgut epithelial cells. Recent work by our group showed that the LdMNPV enhancin genes have an additional function beyond increasing penetration of the peritrophic matrix to virions.

Poster/Virus - Monday, 16:30 - 17:30 V-8

Leafhopper infecting rhabdovirus: Taastrup-like virus.

Wayne Hunter - USDA-ARS, Fort Pierce, Florida, USA; ***Scott Adkins*** - USDA-ARS, Fort Pierce, Florida, USA
Address for correspondence: wayne.hunter@ars.usda.gov

A new viral pathogen of leafhoppers was discovered. The unclassified virus, is a negative sense, single-stranded RNA virus, which appears to be a new member of the order *Mononegavirales*, in the family Rhabdoviridae, and thus far is the first member identified in North America which is related to a recently described Taastrup Virus, also an unclassified member in the Rhabdoviridae. The virus was isolated from an ornamental plant, and when applied to leafhopper cell cultures caused rapid and severe cell death. Current efforts to propagate and sequence the virus will provide a more solid taxonomic classification. New discoveries of insect infecting viruses continues to expand virus taxonomy, as well as advancing the potential biological control agents which may be applied in the management of severe agricultural crop pests, like leafhoppers to reduce Pierce's Disease.

Poster/Virus - Monday, 16:30 - 17:30 V-9

Iridescent virus infection in glassy-winged sharpshooter *(Homalodisca vitripennis*: Hemiptera).

Wayne Hunter - USDA-ARS, Fort Pierce, Florida, USA; ***Ute Albrecht*** - USDA-ARS, Fort Pierce, Florida, USA
Address for correspondence: wayne.hunter@ars.usda.gov

Discovery of leafhopper viral pathogens have increased over the past few years. Leafhoppers transmit many economically important plant pathogens, like the bacteria *Xylella fastidiosa*, which causes Pierce's Disease of grapes which threatens the US viticulture industry. The glassywinged sharpshooter, GWSS, the primary vector of Pierces Disease has a large host range, is larger in size and has the ability to fly long distances. GWSS has also been shown to transmit multiple strains of *Xylella*. GWSS adults were successfully infected with two iridescent viruses: Whitefly Iridovirus, WFIV, and IIV6, both of which had been propagated in *Trichoplusia ni* larvae. Virus infection caused reduced longevity, infected GWSS died an approximately 5 days earlier than non-infected, which resulted in an overall reduction in egg mass production, and fewer nymphs. Nymphal emergence and survivorship were not measured. Adults were infected by microinjection and sprays. Infected individuals transmitted the virus to 'healthy' cohorts when caged together, suggesting an aerosol or sexual mode of transmission. Detection of virus positive eggs suggests that the IIV's may also have a transovarial mode of transmission. Leafhopper vectors of Pierce's Disease, such as the Glassywinged sharpshooter, *Homalodisca vitripennis*, are susceptible to infection by iridescent insect viruses which can decrease sharpshooter survival.

Poster/Virus - Monday, 16:30 - 17:30 V-10

Viral pathogens in leafhopper vectors of Pierce's disease.

Wayne Hunter - USDA-ARS, Fort Pierce, Florida, USA; ***Mizuri Marutani-Hert*** - USDA-ARS, Fort Pierce, Florida, USA; ***Laura Hunnicutt*** - North Carolina State University
Raleigh, North Carolina, USA
Address for correspondence: wayne.hunter@ars.usda.gov

Several newly discovered viral pathogens in leafhoppers have been shown to replicate in two sharpshooter leafhoppers; the glassy-winged sharpshooter, GWSS, *Homalodisca vitripennis*, and *Oncometopia nigricans* (Hemiptera: Cicadellidae). Viruses discovered thus far were classified as members of the Reoviridae, Phytoreoviridae, Rhabdoviridae, and Dicistroviridae. Leafhoppers appear to be permissive to both ssRNA and dsRNA viruses. Two of the viral genomes have been sequenced, and the path of infection into the leafhoppers was determined to be through midgut tissues for members of the Dicistroviridae. The virus occurs naturally in the wild and may provide new agents for biological control strategies in the management of leafhoppers. Currently viral biological control agents of leafhoppers are lacking. The GWSS, is considered the main vector of plant diseases such as Pierce's disease of grapes. But, all leafhoppers tested so far have shown the ability to spread these bacteria, *Xylella fastidiosa*, which causes'Scorch'-like diseases in grapes and other woody crops. These leafhopper-infecting viruses were shown to be infecting field populations of GWSS across several different states from Florida to California. Infected adults were dissected and examined. Analysis of genetic sequences from the salivary glands, and midguts showed high levels of the virus in midgut tissues. Examination by electron microscopy supported midgut tissues as an entry site. Viral pathogens serve to reduce insect populations in nature. Cost/benefit analyses are being conducted on virus mass production and application of leafhopper viruses as a means to reduce the transmission of plant diseases.

Poster/Virus - Monday, 16:30 - 17:30 V-11

Bioaerosols: Insect transmitted pathogens.

Wayne Hunter - USDA,ARS, Fort Pierce, Florida, USA; ***Cindy McKenzie*** - USDA,ARS, Fort Pierce, Florida, USA; ***Bailey Mitchell*** - Electrostatic Space Charge Systems, LLC, Atlanta, Georgia, USA
Address for correspondence: wayne.hunter@ars.usda.gov

Begomovirus was detected as a bioaerosol. Detection of pathogens is critical to monitoring their distribution and spread, and is a key component in the prediction and management of disease epidemiology. Monitoring for pathogens as bioaerosols, requires developing techniques which are sensitive, affordable, and time saving before they will have widespread impact. This approach also overcomes private property issues which are a major pitfall in monitoring diseases in complex agricultural and urban settings. In this study, we have applied an emerging technology of electrostatic sampling to the detection of an insect-transmitted plant pathogen as a bioaerosol. Where insects aggregate in large numbers, as with whiteflies, leafhoppers, psyllids and honey bees, the pathogen (ie. virus or bacteria) becomes aerosolized as thousands of excreta droplets fall from the plants during feeding. Agricultural systems have not fully measured the impact of bioaerosols on disease epidemiology. Electrostatic sampling provides a valuable, affordable, method for monitoring for diseases as bioaerosols which includes plant, animal and human pathogens. Our results successfully used an electrostatic sampling device to collect an aerosolized begomovirus from the air near whiteflies feeding on virus-infected tomato plants.

Poster/Virus - Monday, 16:30 - 17:30 V-12

Metagenomics of leafhoppers and psyllidspsyllids: Discovery of bacteriophages.

Wayne Hunter - USDA-ARS, Fort Pierce, Florida, USA; ***Robert Shatters, Jr.*** - USDA-ARS, Fort Pierce, Florida, USA
Address for correspondence: wayne.hunter@ars.usda.gov

A metagenomics approach was used to determine the presence of bacteriophages in the glassy-winged sharpshooter, GWSS, *Homalodisca vitripennis* (Hemiptera: Cicadellidae) and the Asian Citrus Psyllid, AsCP, *Diaphorina citri* (Hemiptera: Psyllidae). Both species showed evidence of bacteriophages, viruses that infect bacteria. Both of these insects transmit plant infecting bacterial diseases, (GWSS spreads, *Xylella fastidiosa* Pierce's disease of grapes- and AsCP transmits *Can. Liberibacter* spp. associated with Huanglongbing in citrus) both have a rich internal microbial fauna which consists of endosymbionts, and other bacteria, which led to the search for bacteriophages within these insects. Mining cDNA libraries made from these insects produced genetic sequences which had significant homology to phages. Interest in phages is growing and new species are discovered every week. Phages may provide new methods for the management of these two insect vectors, thus reducing the negative impacts of the diseases they transmit in grapes, citrus and other woody agricultural crops.

Poster/Virus - Monday, 16:30 - 17:30 V-13

Enhancing the multiplication of NuclepHPH optimization.

Agata Jakubowska - University of Valencia, Burjassot, Valencia, Spain; ***Juan Ferré*** - University of Valencia, Burjassot, Valencia, Spain; ***Salvador Herrero, Burjassot*** - University of Valencia, Valencia, Spain
Address for correspondence: agatajak@gmail.com

Insect nucleopolyhedroviruses (NPV) are widely studied as agents for biological control, as expression vectors for heterologous proteins production, and as transduction vectors for gene therapy applications. Most of these applications rely on the existence of cell cultures that allow the *in vitro* multiplication of the virus. To date more that 500 continuous cell lines have been established from more that 100 insect species and are daily used in physiology, pathology and molecular biology studies. Efficient infection and multiplication of baculovirus in cell culture determines the success of heterologous protein production and is as well essential for functional studies of baculovirus genes and any other applications involving virus replication. We have explored the influence of medium pH in the multiplication of the Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV), Helicoverpa armigera single nucleopolyhedrovirus (HaSNPV) and Aurographa californica MNPV (AcMNPV) in different cell culture lines. Our studies has revealed a strong influence of the medium pH on the virus multiplication with the best results at pH=6.5, about half unit of pH above the most commonly used insect culture media. Additional experiments using a recombinant AcMNPV carrying the expression of the green fluorescent protein, have revealed that the enhancement of virus multiplication at pH=6.5 is mainly due to a better entrance of the budded virions into the cells. According to these results we recommend to study the optimal pH conditions for each virus-cell line combination for the optimization of baculovirus multiplication in vitro. Additionally, our results have also pointed out the pH manipulation of insect's hemolymph as a possible mechanism of insect response or tolerance to baculoviruses.

Poster/Virus - Monday, 16:30 - 17:30 V-14-STU

Production of a full length cDNA clone and infectious transcripts of deformed wing virus.

Seth Levy - University of Massachusetts – Amherst, Amherst, Massachusetts, USA; ***Woojin Kim*** - University of Massachusetts – Amherst, Amherst, Massachusetts, USA; ***John Burand*** - University of Massachusetts – Amherst, Amherst, Massachusetts, USA
Address for correspondence: jburand@microbio.umass.edu

Bee viruses are receiving attention for the pivotal role they play in the deteriorating health of the European honeybee, *Apis mellifera*. *Iflaviruses* such as Israeli acute paralysis virus (IAPV), which is considered to be a major contributing factor to colony collapse disorder (CCD), are the main focus of the emerging bee virus field. A related *Iflavirus*, Deformed wing virus (DWV), has been found to be present in more than 90% of bees sampled from hives in Massachusetts, yet little is known about the molecular biology of these positive-sense, single-stranded RNA viruses. In an attempt to create infectious DWV transcripts, we constructed full length cDNA clones of the genome of this virus isolated from infected honeybees. This cDNA was used to create infectious RNA transcripts, which were found to replicate when injected into live bumblebees, *Bombus impatiens*. These results clearly indicate that DWV is infectious for both species of bees and suggest that the transmission of these viruses between bee species may occur in the field; identifying a potential threat to native bumble bee populations sharing foraging sites with infected honeybees.

Poster/Virus - Monday, 16:30 - 17:30 V-15

Improved procedures for identification of *Orcytes nudivirus* disease in the Pacific region.

Sean Marshall - AgResearch, Lincoln, New Zealand; ***Jana Monk*** - AgResearch, Lincoln, New Zealand; ***Shareen Prasad*** - Secretariat of the Pacific Community, Suva, Fiji; ***Nicola Richards*** - AgResearch, Lincoln, New Zealand; ***Trevor Jackson*** - AgResearch, Lincoln, New Zealand
Address for correspondence: sean.marshall@agresearch.co.nz

The introduction of *Oryctes* nudivirus (OrNV) in the Asia/Pacific region during the 1960s and 70s to control the rhinoceros beetle (*Oryctes rhinoceros*) led to dramatic reduction in palm damage in many areas, and has been a major success for classical biocontrol with a virus. Recently, control seems to have diminished with outbreaks of the beetle

being reported from some Pacific Islands. Although the reason for this is unknown, evidence suggests the virus is still present in the region. Improved diagnostic techniques are required to reliably identify OrNV diseased rhinoceros beetles from collection sites. Diagnostic techniques have been investigated to improve reliable identification of OrNV diseased rhinoceros beetles collected from sampling sites. The combined use of PCR and histopathology methods were found to be the most reliable procedure for confirming OrNV disease in rhinoceros beetles. PCR based screening was useful for identifying samples negative for the presence of OrNV, but the sensitivity of this method makes it difficult to reliably distinguish diseased beetles from OrNV contaminated samples. Visual inspection of histological sections was a complimentary method to assist in identifying the mid to late stages of OrNV infection, but it lacked some degree of sensitivity in identifying early stages of infection. We have also begun to investigate conditions necessary to maintain viral infectivity in an effort to help improve beetle control in reported outbreak areas. Findings will be discussed in relation to the continued use of this virus for control of the rhinoceros beetle.

Poster/Virus - Monday, 16:30 - 17:30 V-16

Melanized encapsulation and apoptosis impact baculovirus infection in gypsy moth *(Lymantria dispar).*

James McNeil - Penn State University, University Park, PA, USA; ***Matthew Gardner*** - Penn State University, University Park, PA, USA; ***Diana Cox-Foster*** - Penn State University,University Park, PA, USA; ***Kelli Hoover*** - Penn State University, University Park, PA, USA
Address for correspondence: jrm418@psu.edu

The gypsy moth, *Lymantria dispar*, exhibits developmental resistance to the baculovirus *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV) within some larval instars. Mortality in mid-fourth instar larvae is almost half that of newly molted fourth instars inoculated with the same dose of virus. This resistance is driven in part by systemic mechanisms, which we hypothesized involves differential immune responses, such as melanized encapsulation, to virally infected tissue. We also hypothesized that apoptosis of infected tracheal epidermal cells occurs and contributes to viral resistance. To determine if melanized encapsulation differed between newly-molted (highly susceptible) or mid-instar (highly resistant) larvae, we implanted agarose plugs embedded with cultured insect cells infected with a recombinant LdMNPV expressing LacZ into larvae of both ages. For each treatment group, we removed the implants from larval cohorts at 24, 48, and 72 h intervals and measured melanization and LacZ signaling of the implants. We observed more targeted melanization and reduced LacZ signaling of implants taken from resistant-aged larvae. To determine if apoptosis of infected tissue occurs and may contribute to viral resistance, we inoculated newly-molted larvae with the same strain of LdMNPV and dissected tracheal elements from larvae at 2, 7, and 9 d post-inoculation. We processed those tissues with a colorimetric TUNEL assay to visualize apoptotic cells. We also measured gene expression of two viral genes, *iap2* and *iap3,* which are thought to inhibit apoptosis of host cells, to determine if they were actually functioning in the host. We detected clear co-occurrence of infected and apoptotic tracheal epidermal cells and showed the expression of *iap3* diminishes over time post-inoculation. These data indicate that melanized encapsulation and apoptosis of infected tissue could contribute to developmental resistance to baculovirus in *L. dispar* .

Poster/Virus - Monday, 16:30 - 17:30 V-17

***In vivo* inhibition of HZ-2V pathology using anti P74 RNAi.**

Justin Nguyen - University of Massachusetts - Amherst, Amherst, Massachusetts, USA; ***Woojin Kim*** - University of Massachusetts – Amherst, Amherst, Massachusetts, USA; ***John Burand*** - University of Massachusetts – Amherst, Amherst, Massachusetts, USA
Address for correspondence: jburand@microbio.umass.edu

Hz-2V is an eveloped, rod-shaped, insect pathogenic DNA virus that resembles baculoviruses. This virus is transmitted during mating with infected, female *Helicoverpa zea* moths being sterile. These sterile females lack ovaries, bursa copulatrix, accessory glands and spermatheca and have grossly deformed and enlarged common and lateral oviducts, which appear as a large "Y-shaped" structure. As with most large DNA viruses pathogenic to insects, *Hz-2V* contains an ortholog of the baculovirus *P-74* gene. *P-74* is a virus structural protein that functions in the viral entry into host cells the essential, first step in the virus infection process. By using an RNAi approach to down regulate the expression of the *Hz-2V P-74* gene, we have reduced the pathology and potentially inhibited the replication of the virus in infected female moths. We hope to use this approach as a tool to elucidate the role other *Hz-2V* genes play in the replication and pathology of this virus.

Poster/Virus - Monday, 16:30 - 17:30 V-18-STU

Generation of BMNPV- resistant BMN cells.

Daisuke Ohtsuka - Laboratory of applied molecular entomology, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan,; ***Ken Sahara*** - Laboratory of applied molecular entomology, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan; ***Shin-ichiro Asano*** - Laboratory of applied molecular entomology, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan; ***Hisanori Bando*** - Laboratory of applied molecular entomology, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan
Address for correspondence: dohtsuka0809@gmail.com

The *Bombyx mori* nucleopolyhedrovirus (BmNPV) is one of the major agents causing the lethal disease "nucleopolyhedrosis" in the silkworm, which causes serious economic loss in sericulture farms. Recently, the transgenic silkworms possessing a transgene designed to transcribe dsRNA for suppressing essential viral genes were generated (Isobe et al., 2004; Kanginakudru et al., 2007). However, the BmNPV-resistance enough in practical use has not been achieved. The moderate BmNPVresistances observed for the transgenic silkworms were highly probably due to insufficient and/or discontinued expression of dsRNA, which could be caused by the tissue-dependent expression of transgenes and BmNPV-induced shut-off of host gene expression. Thus, one issue to be solved first is a tissue-specific regulation of the promoter used to express dsRNA since it is well established that the gene expression by pol II promoters is tissue-specific *in vivo*. To solve this problem, we developed a small-hairpin RNA (shRNA) expression units driven by a *Bombyx* U6 (BmU6) promoter, a RNA polymerase III (pol III) promoter, which was expected to constitutively express shRNA in a tissue-nonspecific manner. Subsequently, we tried to solve the second problem, ie. the suppression of transgene by BmNPV-induced host shut-off mechanism. Interestingly, viral gene expression is not shut-off during viral replication, indicating some kind of viral mechanisms to escape virus-induced shut-off. We here examined the possibility to use the BmNPV enhancer element *hr5* for keeping or enhancing expression of transgene during viral replication. We then generated BmNPV-resistant BmN cells using the BmU6-shRNA expression system in combination with *hr5* .

Poster/Virus - Monday, 16:30 - 17:30 V-19-STU

Analysis of ACMNPV immediate-early gene knockout viruses – with a focus on the function of ME53.

Chikako Ono - Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan; ***Shin-ichiro Asano*** - Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan; ***Ken Sahara, Sapporo*** - Graduate School of Agriculture, Hokkaido University, Hokkaido, Japan; ***Hisanori Bando*** - Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan
Address for correspondence: chikaono@abs.agr.hokudai.ac.jp

Baculoviral genes are expressed in a stage-dependent manner. Immediate-early genes (ie-genes) are transcribed immediately after infection without requirement of viral transregulators for efficient expression and include the transregulators involved in the regulation of the replication cascade. To understand viral replication mechanisms, it is important to have deep insight into the gene expression network started with the ie-gene expression. We constructed ie-gene-knockout AcMNPVs (KOVs) for each ie-gene (*ie1*, *ie0*, *ie2*, *pe38* and *me53*) using the bacmid system and investigated their growth properties including ie-gene expression profile. In Sf9 cells transfected with the KOV DNAs, we observed the knockout gene-specific positive/negative effects on the expression of ie-genes. Subsequent transient experiments using the ie-gene expression plasmids and the reporter plasmids driven by the ie-gene promoter provided the results consisting with the observations for the KOVs, implying importance of the mutual regulation among ie-genes. Focusing on a frontier ie-gene me53, the knockout of *me53* was resulted both in upregulation of *ie2* and in lack of the productivity of infectious budded virus (BV), implying the possibility that *me53* was involved in the production of infectious BV through the regulation of *ie2*. We here present more data concerning the function of *me53* in BV production.

Poster/Virus - Monday, 16:30 - 17:30 V-20

Protein expression profiles of permissive, semipermissive and non-permissive cells infected by baculovirus.

Holly Popham - USDA ARS BCIRL, Columbia, MO, USA; ***Cynthia Goodman*** - USDA ARS BCIRL, Columbia, MO, USA; ***James Grasela*** - USDA ARS BCIRL, Columbia, MO, USA
Address for correspondence: holly.popham@ars.usda.gov

Amassing information on the *in vitro* protein expression of an insect host challenged by an entomopathogenic agent, such as a baculovirus, is paramount to an enhanced understanding of how host-pathogen interactions determine the success or failure of a pathogen. In this study, 2D-gel electrophoresis and subsequent mass spectrometric analysis employing MALDI TOF/TOF were used to investigate the protein expression of three insect cell lines exposed to baculoviruses for 24 h, including (1) *Heliothis virescens* cells permissive to an *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) challenge, (2) *H. virescens* cells semi-permissive to *Helicoverpa zea* single nucleopolyherovirus (HzSNPV) infection, and (3) *H. zea* cells non-permissive to AcMNPV infection. We found a major shift in the pattern of protein expression between mock-treated controls and *H. virescens* cells exposed to AcMNPV (permissive) as well as HzSNPV (semipermissive). Of the 24 proteins identified in the *H. virescens* mock-treated controls, 21 were either completely absent or present at extremely low expression levels in the AcMNPV infected cells. The three remaining proteins showed an increase in expression levels 2- to 4.3-fold greater than in mock-treated controls. Two of these proteins were identified as signal transduction proteins and one as a DNA supercoiling factor. In the semi-permissive system, changes in protein expression were also found that were suggestive of the protein expression observed in the permissive system. In contrast, the number and expression levels of identified *H. zea* cell proteins exposed to the non-homologous AcMNPV after 24 h showed little change from mock-infected controls. This is somewhat expected as it is based on the nature of the relationship revealed by *in vitro* and *in vivo* studies between the non-permissive *H. zea* cells challenged with AcMNPV.

Poster/Virus - Monday, 16:30 - 17:30 V-21

Functional analysis of the inhibitor of apoptosis genes in antheraea pernyi nucleopolyhedrovirus.

Yipeng Qi - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Songya LV*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Feng Yan*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Hua Xu*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Daqin Zhang*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University Wuhan, Hubei, China
Address for correspondence: qiyipeng@whu.edu.cn

The inhibitor of apoptosis proteins (IAP) play important role in cell apoptosis.We cloned two novel IAP family members, Ap-iap1 and Ap-iap2, from *Antheraea pernyi* Nucleopolyhedrovirus(ApNPV) genome. Ap-IAP1 contains two baculoviral IAP repeat (BIR) domains followed by a RING domain, but ApIAP2 has only one BIR domain and RING. The result of transient expression in Spodoptera frugiperda(Sf21) showed that Ap-iap1 blocked cell apoptosis induced by actinomycin D treatment and also rescued the p35 deficient *Autographa californica* nucleopolyhedrovirus (AcMNPV) to replicate in Sf9 cells,while Ap-iap2 has no these function. Several Ap-IAP1 truncations were constructed to test the activity of BIRs or RING motif to inhibit cell apoptosis. The results indicated that BIRs or RING of App-IAP1 had equally function to inhibit cell apoptosis,therefore deletionof above both domains could not block apoptosis induced by actinomycin D or rescue the replication of AcMNPV?p35. In order to test if IAPs inhibit caspase-3 like activity, we first expressed and purified Ap-IAP1 protein and Ap-IAP2 protein. The result showed that Ap-IAP1 could not inhibit caspase-3 like activity in vitro.

Poster/Virus - Monday, 16:30 - 17:30 V-22

Recombinant expression of the structural proteins of *Porcine* reproductive and respiratory syndromevirus using BMNPV.

Soo-Dong Woo - Chungbuk National University, Cheongju, Chungbuk, Korea; ***Jeong-Mi Oh,*** - Chungbuk National University; ***Hyun-Na Koo,*** - Chungbuk National University; ***Yeon-Ho Je,*** - Seoul National University; ***Byung-Rae Jin,*** - Dong-A University
Address for correspondence: sdwoo@cbnu.ac.kr

The porcine reproductive and respiratory syndrome virus (PRRSV) has six structural proteins which encoded by ORFs 2 to 7 are designated as GP2, 3, 4, 5, M and N, repectively. In this study, we determined the expression of each protein using novel transfer vector, pBmKSK4 which has the polyhedrin promoter of BmNPV and 6xHis tag. The recombinant transfer vector was co-transfected into Bm5 cells along with bBpGOZA DNA. Recombinant virus was purified by plaque assay and amplified in Bm5 cells. Expression of each protein was identified by SDS-PAGE and Western blot analysis using anti-6xHis monoclonal antibody. The expression levels of the structural proteins in Bm5 cells

were stronger than the expression system using pBacPAK9 transfer vector in Sf21 cells. As expected, GP5 was expressed at low levels from its structural properties and its toxicity for cells. In order to enhance the expression of recombinant proteins, we used a SUMO fusion system. The fusion of a PRRSV protein to SUMO increased expression levels as well as enhanced solubility of the recombinant protein. The results of this study have implications for both the taxonomy of PRRSV and vaccine development.

Poster/Virus - Monday, 16:30 - 17:30 V-23-STU

Host RNA polymerase-dependent transcription strategies of insect viruses.

Jianli Xue - Miami University, Oxford, Ohio, USA; ***WeiYing Wang*** - Miami University, Oxford, Ohio, USA; ***Colin Turney*** - Miami University, Oxford, Ohio, USA; ***Xiao-Wen Cheng*** - Miami University, Oxford, Ohio, USA
Address for correspondence: xuej@Muohio.edu

Ascovirus and baculovirus are both insect specific viruses that have the potential for insect pest control. Genetic and molecular data predicted that ascovirus is closely related to nuclear replicating baculovirus which depends on host RNA polymerase for early gene transcription. Ascovirus has 3 homologues to RNA polymerase II. *In vitro* transcription with the nuclear extract of Sf21 cells showed that *ie1* and *lef-8* early gene promoters and the late polyhedrin gene promoter of Autographa californica MNPV (AcMNPV) as well as the RNA polymerase homologue gene (*ORF 126*) promoter of ascovirus can be recognized by host RNA polymerases, whereas the late mcp gene promoter of Spodoptera frugiperda ascovirus (*SfAV-1a*) is not recognized by host RNA polymerases. Canonical host polyadenylation signal AAUAAA is found in most baculovirus late genes, but not in the early genes. No obvious host polyadenylation signal was found in early and late genes of ascovirus. Previous studies showed that late genes of baculovirus and late gene mcp of ascovirus are polyadenylated. 3' RACE results showed that ie-1 and *ORF126* are not polyadenylated. Amanitin treatment only partially inhibited AcMNPV infection in *Sf21*. All these results suggest that RNA polymerase II may not be responsible for all the baculovirus and ascovirus early gene transcription.

Poster/Virus - Monday, 16:30 - 17:30 V-24

A recombinant *Anticarsia gemmatalis* multiple nucleopolyhedrovirus with interruption of the *pif-1* gene.

Briana Cardoso Ferreira - Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil; ***Maria Elita Batista de Castro*** - Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil; ***Francisco José Rivera Pinedo*** - Faculdade Juscelino Kubitschek, Brasília, DF, Brazil; ***Marlinda Lobo de Souza*** - Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil
Address for correspondence: marlinda@cenargen.embrapa.br

Baculovirus are pathogenic viruses to arthropods and infect mainly insects of the Lepidoptera order. Its main route of infection is through the ingestion of viral particles by the host larvae. Baculovirus possesses a peculiar feature of a two phenotype formation during its infection cycle: the Occlusion Derived Virions (ODV), responsible for the initial entrance of the virus in the caterpillar intestine epithelium, and the Budded Virus (BV), involved in the systemic infection of host larvae tissues. The ODV entrance in the epithelial cells is mediated by the P74 protein and by proteins of the pif genes family (per os infectivity factor), named pif-1, pif-2 and pif-3. The pif genes are essential in the oral infection process once in the absence of any of them the disease does not occur. The Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV)is widely used in Brazil for the soybean caterpillar control. With the aim to study the function of the AgMNPV pif-1 gene the construction of a recombinant virus with interruption of this gene was made, by inserting the gfp reporter gene in the pif-1 gene locus, after a homologous recombination assay. In that way, a co-transfection in BTI-Tn-5B1-4 insect cells was made, using a plasmid containing the gfp gene, flanqued by the homologous regions to the pif-1 gene, under the constitutive hsp70 promoter expression, and the AgMNPV virus genomic DNA. It was possible to detect the presence of the recombinant virus in cells, by observing fluorescence under a phase contrast microscope with UV light due to the GFP protein expression. Clones of the recombinant virus (vAgGFP?pif-1) with the pif-1 gene inactivated were selected by serial dilution technique.

Poster/Virus - Monday, 16:30 - 17:30 V-25

Direct fusion is an alternative pathway for ACMNPV to efficiently gain entry into insect and mammalian cells.

Sicong Dong - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Manli Wang*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Fei Deng*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Zhihong Hu*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Hualin Wang*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China
Address for correspondence: h.wang@wh.iov.cn

Direct fusion is an alternative pathway for AcMNPV to efficiently gain entry into insect and mammalian cells. It has been demonstrated that the budded virus (BV) of the Autographa californica nucleopolyhedrovirus (AcMNPV) gains entry into insect and mammalian cells through the clathrin-dependent endocytosis pathway. However, in this study, we show that exposure of the budded virus (BV) to acid medium allows it to gain entry into cells in the presence of inhibitors of clathrin-dependent endocytosis pathway. This suggests that BV could enter insect cells and cause infection by direct fusion of viral envelop to cell membranes. We also demonstrated that the infection efficiency is comparable to virus using the endocytosis pathway. We further showed that the myosin-like proteins were essential for nucleocapsid transport and that intact microtubules did not play a role in this process. In the case of mammalian cells, attached virus eventually gained entry under acid conditions even in the presence of ammonium chloride. Furthermore, it was quite evident that this particular acid treatment significantly increased the transduction rate in mammalian cells suggesting that the treatment of BVs at low pH would be quite useful in the transduction of mammalian cells with baculoviruses.

Poster/Virus - Monday, 16:30 - 17:30 V-26

The CPGV spindlin-like protein enhanced the infectivity of baculoviruses and Bacillus thuringiensis.

Xiangyang Liu - Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, China; ***Xiulian Sun*** - Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, China
Address for correspondence: sunxl@wh.iov.cn

A codling moth (Cydia pomonella) outbreak occurred in the Xinjiang Uygur Autonomous Region of China in 1980's and spread to Gansu Province in 1992. It became one of the most serious insect pests of apples and pears in the area in recent years. C. pomonella granulovirus (CpGV) is being used as commercial bioinsecticides in a number of countries but not in China. CpGV orf13 encodes a homologue of baculovirus GP37

(spindlin) that has been proposed to enhance the infectivity of some viruses as it shared high amino acid sequence identity and conserved domains with the entomopoxvirus infectivity enhancer (fusolin). We report here that the production of Cp13 expressed in E. coli might enhance the infectivity of both Autographa californica MNPV (AcMNPV) and Bacillus thuringiensis in 3rd instar Spodoptera exigua larvae. The median lethal concentration (LC_{50}) of AcMNPV was reduced 14.1 times when the expressed spindlin-like protein was combined at a rate of 30 µg/ml in the virus suspensions. This synergistic ratio was similar to that (12.0 times) of the enhancin from Agrotis segeturn granulovirus. When the spindlin-like protein was combined with a sensitive strain (LC^{50} = 4.9 µg of solubilized protoxin/ml) and a less sensitive strain (LC^{50} = 17.7 µg of solubilized protoxin/ml) of B. thuringiensis, the synergistic ratio was 2.5 and 3.8 times, respectively. These results provide a potential to develop effective and low-cost formulations by combining the Cp13 with B. thuringiensis for the control of the codling moth.

Poster/Virus - Monday, 16:30 - 17:30 V-27

Metagenomics approach to discover virus: Diaphorina citri reovirus.

Mizuri Marutani-Hert - USDA-ARS, Fort Pierce, Florida, USA; ***Wayne Hunter*** - USDA-ARS, Fort Pierce, Florida, USA; ***David Hall*** - USDA-ARS, Fort Pierce, Florida, USA
Address for correspondence: wayne.hunter@ars.usda.gov

A metagenomics approach lead to discovery and characterization of a new viral pathogen of Asian citrus psyllid, *Diaphorina citri*, Kuwayama (Psyllidae: Hemiptera), as a member of Reoviridae. We produced a cDNA library from adult psyllids to discover new pathogens that may be use for psyllid biological control. We identified viral sequences with significant similarity to insect Reovirus. Multiple sequence alignments of predicted amino acid sequences resulted in 48% and 30%, shared identity to RNA polymerase and S2 segment of NLRV, respectively, with 25% identity to 'B' spike structural protein of Fiji Disease Virus, FDV, and 20% identity to an unnamed protein product of *Diadromus pulchellus idnoreovirus 1* (DpRV). To confirm the incidence of psyllids infected by Reovirus, psyllids were assayed for the virus by RT-PCR. Psyllids which were collected from the field (May 2008) resulted in ~55% virus positive. Virus acquisition and transmission may be occurring due to a combination of the *D. citiri* feeding behavior and wide host range overlap with Reovirus host plants. The virus was also determined to consist of subgenomic strands similar to members of the Fijivirus'. Phylogenetic and homology comparisons indicated that the viral sequences were most closely related to the viruses in the Family Reoviridae, Genus *Fijivirus*, specifically *Nilaparvata lugens reovirus* (NLRV).

Poster/Virus - Monday, 16:30 - 17:30 V-28

An active DNA photolyase (AMV025) from *Amsacta moorei* entomopoxvirus.

Remziye Nalcacioglu - Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, Trabzon, Turkey; ***Kazim Sezen*** - Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, Trabzon, Turkey; ***Ikbal Agah Ince*** - Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, Trabzon, Turkey; ***Just M. Vlak*** - Laboratory of Virology, Wageningen University, Sault Ste. Marie, Ontario, Canada; ***Zihni Demirbag*** - Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, Wageningen, the Netherlands; ***Basil Arif,*** - Great Lakes Forestry Centre, Canadian Forest Service; ***Monique M. van Oers*** - Laboratory of Virology, Wageningen University,
Address for correspondence: monique.vanoers@wur.nl

The major damage induced in DNA by ultraviolet light is the induction of cyclobutane pyrimidine dimers (CPDs). These dimers can be reverted into monomers by a blue light dependent reaction catalyzed by enzymes called DNA photolyases. Specific DNA photolyases have been detected in a wide variety of organisms ranging from bacteria to non-placental mammals. *Amsacta moorei* entomopoxvirus (AmEPV) is one of a few viruses that carry a photolyase gene. The genome of AmEPV has been entirely sequenced and 279 open reading frames (ORF) have been predicted. Among these ORFs, AMV025 has a 1362 nt coding sequence for a putative photolyase protein of 453 amino acids. The predicted amino acid sequence includes a FAD binding domain and a region similar to a DNA photolyase domain. Transient expression of the AMV025 gene in an *Escherichia coli* strain lacking the endogenous photolyase enzyme showed rescue of bacterial growth after UV irradiation in the presence of blue light, indicating that AMV025 encodes an active DNA photolyase. When compared with the *phr2* gene of the baculovirus *Chrysodeixis chalcites* NPV, AMV025 showed a reduced activity. Amino acid alignment of the AmEPV photolyase with other photolyases from poxviruses and baculoviruses resulted in amino acid identities ranging between 27 and 39%. Further studies will reveal whether the AmEPV photolyase protein is present in AmEPV virions. In the related fowlpox virus, a DNA photolyase is present in the virions and repairs UV induced lesions.

Poster/Virus - Monday, 16:30 - 17:30 V-29

Bacterial but not baculoviral infections stimulate hemolin expression in *Helocoverpa zea* and *Heliothis virescens*.

Olle Terenius - Global and Swedish Research Cooperation, Stockholm, Uppsala, Sweden; ***Holly Popham*** - USDA ARS BCIRL, Columbia, MO, USA; ***Kent Shelby*** - USDA ARS BCIRL, Columbia, MO, USA
Address for correspondence: holly.popham@ars.usda.gov

Lepidopteran larvae are regularly infected by baculoviruses during feeding on infected plants. The differences in sensitivity to these infections can be substantial, even among closely related species. For example, the noctuids Cotton bollworm (*Helicoverpa zea*) and Tobacco budworm (*Heliothis virescens*), which a couple of decades ago were considered to belong to the same genus, have a 1000-fold difference in sensitivity to *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) infection. Recent data were interpreted to indicate that the lepidopteran immunoglobulin protein, Hemolin, is activated upon viral injection and therefore to participate in anti-viral responses. To investigate whether Hemolin is activated after a natural virus infection specific transcription in fat bodies and hemocytes of *H. zea* and *H. virescens* larvae was monitored following *per os* infection with the baculovirus HzSNPV (*H. zea* single nucleopolyhedrovirus). Both moths showed strong Hemolin expression at 24 and 48 hours, which are time points that correspond to viral entry into hemocytes. However, the same expression pattern was seen in uninfected animals and coincided with ecdysone responses, previously known to induce Hemolin expression. In contrast, injection of lyophilized *Micrococcus luteus* resulted in increased Hemolin expression supporting a role for Hemolin as an immunoresponsive protein in these species. The combined data are consistent with the suggestion that while Hemolin seems to participate in the response to virus infection in the superfamily Bombycoidea, this is not true in the Noctuoidea.

Poster/Virus - Monday, 16:30 - 17:30 V-30

Integration of picorna-like viruses in multiple insect taxa.

Danielle Tufts - University of Texas at Tyler, Tyler, Texas, USA; ***K Spencer*** - University of Texas at Tyler, Tyler, Texas, USA; ***Wayne Hunter*** - USDA-ARS, Fort Pierce, Florida, USA, ***Daymon Hail*** - University of Texas at Tyler, Tyler, Texas, USA; ***Blake Bextine*** - University of Texas at Tyler, Tyler, Texas, USA
Address for correspondence: wayne.hunter@ars.usda.gov

The Picornaviridae superfamily consists of over 450 species of positive single stranded RNA viruses. This family is unique in that all members have a protein that is attached to the 5' end which is used as a primer for RNA polymerase during transcription. Picorna viruses infect many different organisms, including mammals, birds, and insects. In this study we provide evidence that picorna-like viruses are present in a range of insect hosts and that this type of virus has integrated into the DNA of various insect species. We provide evidence of picorna-like viruses in the glassywinged sharpshooter (*Homalodisca vitripennis*), HoVV; in the red imported fire ant (*Solenopsis invicta*), SINV; and in the European honeybee (*Apis mellifera*). Analysis of reverse transcriptase PCR (RT-PCR) demonstrated that viruses in the subgroup Dicistroviridae have integrated into the genomes of *S. invicta* and *A. mellifera*. However, integration of the leafhopper infecting virus, HoVV-1 into the glassy-winged sharpshooter, *H. vitripennis* was not detected.

Poster/Virus - Monday, 16:30 - 17:30 V-31

Identification of a nucleopolyhedrovirus in winter moth populations from Massachusetts.

Anna Welch - University of Massachusetts - Amherst, Amherst, Massachusetts, USA; ***George Boettner*** - University of Massachusetts – Amherst, Amherst, Massachusetts, USA; ***Vincent D'Amico*** - USDA-Forest Service, University of Delaware, Newark, Delaware, USA; ***Joseph Elkinton*** - University of Massachusetts - Amherst, Amherst, Massachusetts, USA; ***John Burand*** - University of Massachusetts - Amherst, Amherst, Massachusetts, USA
Address for correspondence: jburand@microbio.umass.edu

The winter moth, *Operophtera brumata*, originally from Europe, has recently invaded eastern Massachusetts. This insect has caused widespread defoliation of many deciduous tree species and severely damaged a variety of crop plants in the infested area including apple, strawberry and especially blueberry. Using PCR with primers designed to amplify a 484 bp region of the baculovirus polyhedrin gene, we were able to identify *O. brumata* nucleopolyhedrovirus (OpbuNPV) infected winter moth larvae collected from field sites in Massachusetts. This represents the first report of OpbuNPV in winter moth populations in the U.S. An analysis of larvae from seven established, winter moth populations in Massachusetts revealed the presence of the virus in two of these populations, with the prevalence of 80% in one population and 25% in the other. Subsequently, using this same approach, we were able to detect viral sequences in winter moth pupae that failed to emerge suggesting that these insects died as a result of viral infection.

Poster/Virus - Monday, 16:30 - 17:30 V-32

Physiological basis for increased AGIPMNPV infection following feeding of *Agrotis ipsilon* larvae on Herculex I corn.

Nina Schmidt - Iowa, Ames, IA, USA; ***Jessica Haywood*** - Iowa State University, Ames, IA, USA; ***Bryony Bonning*** - Iowa State University, Ames, IA, USA
Address for correspondence: bbonning@iastate.edu

Larvae of the black cutworm, *Agrotis ipsilon* Hufnagel, were more susceptible to infection by *Agrotis ipsilon* multiple nucleopolyhedrovirus (AgipMNPV, Baculoviridae) after feeding on Herculex I (Dow AgroSciences LLC), a transgenic corn hybrid expressing the *Bacillus thuringiensis* (Bt) toxin Cry1Fa2 compared to larvae fed on isoline corn. We investigated the physiological basis for increased susceptibility to virus infection following exposure to Herculex I with emphasis on factors important for both Bt Cry toxin action and baculovirus infection, namely midgut pH, gut protease activity and peritrophic membrane structure. There were no significant treatment differences in midgut pH in larvae fed Herculex I or isoline diet. Membrane associated aminopeptidase activity and soluble chymotrypsin-like proteinase activity were significantly lower in Herculex I -fed larvae, compared to isoline-fed larvae, although the number and relative molecular masses of soluble chymotrypsin-like proteinases did not differ between the two treatments. In vitro analysis indicated that baculovirus polyhedra are not susceptible to degradation by chymotrypsin. From SEM analysis of the peritrophic matrices of Herculex I -fed larvae and isoline-fed larvae we concluded that Herculex I did not damage the peritrophic matrix, which could facilitate subsequent baculovirus infection. Further analyses are required to determine whether Bt toxin-induced epithelial cell sloughing would enhance subsequent virus infection.

Beneficial Division Workshop Wednesday, 20:00 - 21:15
White Pine I-II

Bee Health—Diseases and Cures

Organizer/Moderator: Elke Genersch

Workshop paper - Wednesday, 20:00 122

RNAi at work in real life application: Targeting invertebrate pests and beneficial organisms' diseases.

Eyal Ben-Chanoch - Beeologics, Miami, FL, USA; ***Nitzan Paldi*** - Beeologics, Rehovot, Israel
Address for correspondence: eyal@beeologics.com

RNA interference (RNAi) is a highly conserved, naturally occurring mechanism of gene silencing found across kingdoms, RNAi is primed when long double-stranded RNA molecules (dsRNA) are recognized and cleaved by the RNase III enzyme "DICER" into short interfering RNAs (siRNA) These siRNAs are incorporated with the aid of DICER into the RNA-induced silencing complex (RISC), which subsequently targets complementary mRNA sequences in the cytoplasm. For example, during cytoplasmic replication of RNA viruses, the replicative forms represent the pool of dsRNAs that trigger induction of the silencing-based, antiviral defense mechanism. Beeologics (www.beeologics.com) has established a simple and relatively inexpensive procedure to produce kilogram quantities of dsRNA homologous to target pest or pathogen sequences. "Remebee" is dsRNA produced in-vitro and is homologous to honeybee viral sequences. The exogenously supplied Remebee mimics the natural dsRNA intermediate involved in viral replication, within the honeybee. In field trials, the gene silencing mechanism induced by Remebee that was fed to the bees was shown to be highly effective in preventing honeybee mortality from the Israel Acute Paralysis Virus (IAPV). Invertebrates provide two types of large scale RNAi application opportunities; pest control and insect health. The former involves sustainable applications to keep pest populations low, and the latter can help keeping beneficial insects healthy. RNAi-based solutions are environmentally friendly, are very specific and can target any organism whose genome has been even partially sequenced. Mechanism of action and some ongoing large-scale projects will be discussed.

Workshop paper - Wednesday, 20:15 123

Parasites in bumble bees - An overview of virulence, epidemiology and the impact of commercial breeding.

Mark Brown - Royal Holloway, University of London, Egham, Surrey, United Kingdom
Address for correspondence: mark.brown@rhul.ac.uk

The impact of parasites – their virulence – is largely determined by their mode of transmission and their life-history. I will discuss these ideas using bumble bees and their microparasites – the trypanosome *Crithidia bombi* and the microsporidian *Nosema bombi* – as examples. Bumble bees are economically and ecologically important pollinators, and therefore understanding their interactions with parasites has significant importance. They have been blamed for the collapse of commercial breeding in the US, and fingered as causes of decline in North American bumble bee populations. I will examine how commercial breeding might affect parasite virulence, and the potential implications of this for natural populations.

Workshop paper - Wednesday, 20:30 124

Honey bee viruses and their impact on honey bee health.

Elke Genersch - Institute for Bee Research, Hohen Neuendorf, Brandenburg, Germany
Address for correspondence: elke.genersch@rz.hu-berlin.de

The honey bee (*Apis mellifera*) is the most important pollinating insect species in agriculture and is, therefore, among the most important productive livestock. However, honey bee diseases and colony losses due to numerous pathogens, like viruses, bacteria, fungi, and metazoan parasites, negatively affect the profitability of agriculture and apiculture. Although honey bee viruses normally cause covert infections without any detectable symptoms, several studies implicate that the combination of certain virus infections and the ectoparasitic mite *Varroa destructor* pose a serious threat to honey bee welfare. We will here give an overview over honey bee viruses with special emphasis on the role of *Varroa destructor* as virus vector.

Workshop paper - Wednesday, 20:45 125

Chalkbrood distribution and transmission in U.S. populations of the alfalfa leafcutting bee.

Rosalind James - USDA-ARS Bee Biology & Systematics Lab, Logan, UT, USA; ***Theresa Pitts-Singer*** - USDA-ARS Bee Biology & Systematics Lab, Logan, UT, USA; ***Ellen Klinger*** - USDA-ARS Bee Biology & Systematics Lab, Logan, Utah, USA

Address for correspondence: rosalind.james@ars.usda.gov

The alfalfa leafcutting bee (*Megachile rotundata*) is highly susceptible to fungi in the genus *Ascosphaera* (Ascomycetes: Ascosphaerales), and infections cause a disease of larvae called chalkbrood. Larvae become infected when they consume spores that have contaminated their food provisions of pollen and nectar, which are gathered by the mother bee. Nine species of *Ascosphaera* are known to infect megachilid bees, but only two species were found infecting alfalfa leafcutting bees in alfalfa seed fields in the western U.S., and nearly all infections were caused by one species, *A. aggregata*. *Ascosphaera proliperda* was the second most common pathogen, sometimes occurring in nearly half the infected larvae, but mainly as co-infections with *A. aggregata*. Nearly all pollen provisions, collected from two areas and throughout the flight season, were contaminated with spores from both pathogens, containing 10^3-10^5 spores per provision. Thus, these fungi are readily spread from dead, sporulating cadavers in one year to pollen provisions of new bees the next year. However, disease incidence is affected by both spore dosage and weather conditions.

Workshop Paper - Wednesday, 21:00 126

The prevalence, distribution and hosts of *Crithidia bombi* in wild bumble bee populations.

James Strange - USDA-ARS-Pollinating Insect Research Unit, Logan, UT, USA; ***Nils Cordes*** - University of Illinois, Illinois Natural History Survey, Urbana, IL, USA; ***Leellen Solter*** - University of Illinois, Illinois Natural History Survey, Urbana, IL, USA; ***Terry Griswold*** - USDA-ARS-Pollinating Insect Research Unit, Logan, UT, USA; ***Sydney Cameron*** - Department of Entomology, and Program in Ecology, Evolution and Conservation Biology, University of Illinois, Urbana, IL, USA
Address for correspondence: james.strange@ars.usda.gov

Several species of bumble bees, predominantly those of the subgenera *Bombus* sensu strict and *Fervidobombus*, are declining across the United States. While the causes of these declines are as yet unknown, pathogen spillover from commercial colonies has been suggested as a possible precipitating factor. One hindrance to further investigation is the paucity of baseline data available on the pathogen complex of U.S. bumble bee populations. The present study targets six *Bombus* species, two declining and four abundant, in 153 sites in 25 western and Midwestern states. Here we report the prevalence of a trypanosome protozoa, *Crithidia bombi* in native bumble bee populations. Our data suggest that *C. bombi* occurs more frequently and at higher prevalence in species of the subgenus *Pyrobombus* than in species from other subgenera; however, there is also a strong spacial component to the prevalence of *C. bombi* infection. *Bombus mixtus*, *Bombus vosnesenskii* and *Bombus bifarius* are the most commonly infected species in the western US, while *Bombus impatiens* is the most commonly infected species in the Midwest. Variability in disease occurrence is strongly correlated with site and cross-infestation of multiple species at a single sight is common.

THURSDAY — 20 August

Symposium (Microbial Control Division) Thursday, 08:00 - 10:00
White Pine I-II

Biopesticides in Organic Farming: Available and Potential Technologies

Organizer/Moderator: Surendra Dara

Symposium - Thursday, 08:00 127

Microbial control of agricultural pests in South Korea.

Jeong Jun Kim - Applied Entomology Division, NAAS, RDA, Suwon, Gyeonggi, Republic of KOREA ; ***Sang Guei Lee*** - Applied Entomology Division, NAAS, RDA, Suwon, Gyeonggi, Republic of KOREA ; ***Siwoo Lee*** - Applied Entomology Division, NAAS, RDA, Suwon, Gyeonggi, Republic of KOREA ; ***Hyeong-Jin Jee*** - Agricultural Microbiology Division, Suwon, Gyeonggi, Republic of KOREA
Address for correspondence: jjkim@rda.go.kr

Microbial control of arthropod pests in Korea started in late 70s to control fall webworm with an entomopathogenic virus. Many studies were conducted to develop microbial pesticides but there were no commercial microbial pesticides developed in Korea until the late 90s. In 2000, the Korean government established guidelines for experimental protocols, requirements and reviews of dossiers for biopesticide registration.

This strengthened research efforts by government research institutes, universities and industry for developing biopesticides. Topseed® based on *Paenibacillus polymyxa*, and Solbichae® based on *Bacillus thuringiensis* subsp. *aizawai*, were the first biopesticides developed and registered in Korea in 2003 to control powdery mildew in cucumber and to control diamond back moth and beat armyworm in Chinese cabbage. The following three biopesticides were developed and are currently registered in Korea : 1) Tobagi®, based on *B. thuringiensis* subsp. *aizawai* to control moths including diamond back moth and oriental tobacco budworm in pepper, rice etc.; 2) Bangsili®, based on *Paecilomyces fumosoroseus* to control greenhouse whitefly and two-spotted spider mite in strawberry and cucumber; and 3) Ddangumi®, based on *Monacrosporium thaumasium* to control root knot nematode in watermelon. There are 5 other Bt products and 2 entomopathogenic fungi based products that are imported for the Korean market. The number of microbial pesticide related patents pending and/or obtained has been increasing especially with a sharp rise after 2000. The share of microbials in the pesticide market was only 0.25% in 2005. This is expected to expand to about 10% or $100M by 2010. I will show successful examples of commercial biopesticides and current research in this area.

Symposium - Thursday, 08:30 128

Dipel® biological insecticide: An integral tool for pest control in organic apple production.

Russ Eldridge - Valent BioSciences, Libertyville, Illinois, USA
Address for correspondence: russ.eldridge@valent.com

The growth in organic produce sales in United States and global markets has been dramatic, with double digit increases for multiple years. Production of organic apples has contributed significantly to this expansion. There are over 150,000 hectares of apples grown in the United States with approximately 3-4% of the hectares devoted to organic production. Apples have a diverse range of insect pests that impact both apple harvest and quality. Leafrollers are a highly problematic pest complex for both conventional and organic apple growers. Conventional growers have a full range of control products for keeping these pests in check while the insecticide tool box for the organic grower is much more limited. Multiple years of field data shows that the *Bacillus thurigiensis kurstaki* product DiPel® is an excellent tool for growers seeking an effective, organic insecticide for control of leafrollers. Use of DiPel® can provide significant control of oblique-banded leafroller (*Choristoneura rosaceana*) and pandemis leafroller (*Pandemis pyrusana*), especially when utilized as an integrated program that includes accurate pest identification, good population assessment, cultural and biological control. In many cases, DiPel® control of oblique-banded leafroller was equivalent to that achieved using conventional chemical standards.

Symposium - Thursday, 09:00 129

Entrust® insecticide: A key tool in organic farming.

Tom Meade - Dow AgroSciences, LLC, Indianapolis, IN, USA; ***Jesse Richardson*** - Dow AgroSciences, LLC, Hesperia, CA, USA; ***Luis Gomez*** - Dow AgroSciences, LLC, Indianapolis, IN, USA; ***James E. Dripps*** - Dow AgroSciences, LLC, Indianapolis, IN, USA; ***Doris Paroonagian*** - Dow AgroSciences, LLC, Indianapolis, IN, USA
Address for correspondence: meade@dow.com

Entrust™ is an organic formulation of spinosad, a mixture of two insecticidal metabolites produced through the aerobic fermentation of the naturally occurring actinomycete *Saccharopolyspora spinosa.* The success of Entrust® insecticide in organic agriculture can be attributed to its many unique attributes. It is a selective insecticide that is highly effective on economically important pests in the orders Lepidoptera, Thysanoptera, Diptera, and Coleoptera but has little or no effect on most beneficial arthropod species. It is fast acting, has both contact and ingestion activity, and controls multiple life stages. Entrust® has a reduced risk profile on non target organisms as well as the environment. Entrust® insecticide is considered a key tool in an integrated approach to insect pest management in organic farming. ™ Trademark of Dow AgroSciences LLC

Symposium - Thursday, 09:30 130

Baculoviruses and fungi as commercial biopesticides: Poised for a breakthrough?

Michael Dimock - Certis USA, Columbia, MD, USA
Address for correspondence: mdimock@certisusa.com

Viral and fungal biopesticides have been seeing increased use as commercial biopesticides in recent years, even as Bt sales have been flat or trending downward. There are signs that this growth will continue and possibly accelerate. Recent and historical developments will be compared for insights into how interacting technological and economic factors have influenced success of these and other microbial products.

Symposium (Microsporidia Division) Thursday, 08:00 - 10:00
Painted Horse I-II

Microsporidia of Beneficial Arthropods

Organizer/Moderator: David Oi

Symposium - Thursday, 08:00 131

***Nosema ceranae* research in Spain: A review**

Raquel Martín-Hernández - Bee Pathology Laboratory, Apicultural Center of Marchamalo, Marchamalo, Guadalajara, Spain; ***Aránzazu Meana*** - Animal Health Dep. Faculty of Veterinary (UCM), Madrid, Madrid, Spain; ***Pilar García-Palencia*** - Medicine and Surgery Dep. Faculty of Veterinary (UCM), Madrid, Madrid, Spain; ***Mariano Higes*** - Bee Pathology Laboratory, Apicultural Center of Marchamalo, Marchamalo, Guadalajara, Spain
Address for correspondence: rmhernandez@jccm.es

Nosema ceranae was first detected in May 2005 in field samples from several geographical areas of Spain while developing molecular diagnosis for *N. apis*. Once detected, pathogenicity was also confirmed to *Apis mellifera* in experimental infections and studies focussed on different fields. Spanish beekeepers had been suffering unusual high colony losses in previous years and research on it was being carried out by Beekeeping Centre of Marchamalo in collaboration with others public organisms. A retrospective analysis of data indicated a loss of seasonality in *Nosema* spore detection and a six-fold higher risk to suffer colony losses in those infected by *N. ceranae*. So it was included in a national survey as well as others factors. Monitoring naturally infected colonies as well as data collected from professional apiaries showed Koch postulates; *N. ceranae* can cause the collapse of infected colonies after more than one year. A long incubation period is characteristic in which no clear signs of disease are easily detected until loss of bees are evident and colony death happens. The presence of brood in winter or a false recovery previous to death are some of them. Experimental infection of bees after empirical observations confirmed the infectivity of corvicular pollen or regurgitated pellets of bee-eaters playing a role on the spreading of infection. The reliable molecular tools developed have also let us to confirm the wide spread of *N. ceranae* either in different countries as in different hosts

as bumblebees. Experimental studies have evidenced great differences between *N. ceranae* and *N. apis* in relation with pathology or endogenous cycles adaptations at different temperatures, immune effect or even host range. Studies confirm *N. ceranae* as an emergent pathogen in honeybee. Acknowledgements: everybody supporting and collaborating with us. INIA-FEDER FOUNDS (RTA2005-00152), MAPYA (API06-009).

Symposium - Thursday, 08:24 132

Nosema ceranae and nosema disease in honey bee.

Wei-Fone Huang - Illinois Natural History Survey, University of Illinois, Champaign, Illinois, United States; ***Leellen Solter*** - Illinois Natural History Survey, University of Illinois, Champaign, Illinois, United States; ***Chung-Hsiung Wang*** - Department of Entomology, National Taiwan University, Taipei, Taiwan
Address for correspondence: wfhuang@illinois.edu

Nosema ceranae is one of two pathogens causing "nosema disease" in honey bees. This microsporidium, relatively recently described, and *N. apis*, known for over 100 years, share characteristics such as sensitivity to fumagillin and similar morphology under light microscopy. Genetic approaches and/or TEM observations are necessary to distinguish these related species. *N. ceranae* is reported to have spread to *Apis* species worldwide and replaced *N. apis* in some areas, even before it was recognized and described in the Asian honey bee in 1996. There remain questions about *N. ceranae* transmission and its replacement of *N. apis*. Symptoms resulting from *N. ceranae* infection that we found were similar to those of *N. apis*, including swollen midgut tissues and crawling behavior, but the bees developed these typical symptoms in only a few sites. The nature of symptoms may be affected by environmental factors or differences among intra-specific strains of *N. ceranae*. We attempted to identify intra-species strains using rDNA and intergenic spacers of the rDNA repeat unit, common molecular markers used for microsporidia. The rDNA and spacer sequences were too conserved to distinguish the strains; however, by comparing rDNA sequences, we found another yet-tobe- identified *Nosema* species.

Symposium - Thursday, 08:48 133

Are microsporidia involved in bumble bee decline?

Nils Cordes - Illinois Natural History Survey, University of Illinois, Champaign, IL, USA; ***Leellen Solter*** - Illinois Natural History Survey, University of Illinois, Champaign, IL, USA; ***Sydney Cameron*** - Department of Entomology, University of Illinois, Urbana, IL, USA; ***Jeffrey Lozier*** - Department of Entomology, University of Illinois, Urbana, IL, USA; ***James Strange*** - USDA-ARS Pollinating Insect Research Unit, Logan, UT, USA; ***Terry Griswold*** - USDA-ARS Pollinating Insect Research Unit, Logan, Utah, USA
Address for correspondence: ncordes@life.illinois.edu

Several species of bumble bees, predominantly those of the subgenus *Bombus* sensu stricto, are rapidly declining in different geographical areas of the United States. The reasons for decline are not yet clear, and recent reports that pathogen spillover from commercial colonies are involved is difficult to substantiate because there is so little baseline data available on the pathogen complex of U.S. *Bombus* populations. We are providing such a data set by conducting comparative surveys and screening for six *Bombus* species, two declining and four stable and abundant species in 153 sites in 25 western and midwestern states. We focused on the microsporidia as a potential exotic invader and show that *Nosema bombi* remains the only microsporidian species recovered from bumble bees worldwide and has a broad ecological host range in North America, as it does in Europe. Our data suggest that *N. bombi* occurs more frequently and at higher prevalence in species of the genus *Bombus* sensu stricto than in species from other subgenera. *Bombus occidentalis*, *Bombus pensylvanicus* (species of concern) and *Bombus mixtus* are the most commonly infected species. Variability in disease occurrence is strongly correlated with site. We are currently conducting experiments on comparative susceptibility of several species.

Symposium - Thursday, 09:12 134

Social parasitism in microsporidia: *Kneallhazia solenopsae* development in fire ant colonies.

Yuliya (Julia) Sokolova - Institute of Cytology Russian Academy of Sciences, St. Petersburg, St.Petersburg, Russia; ***James Fuxa*** - Louisiana State University AgCenter Entomology Dept., Baton Rouge, LA, USA
Address for correspondence: jumicro@yahoo.com

Recently we created a new genus and a new combination *Kneallhazia solenopsae* for the fire ant parasite *Thelohania solenopsae* Knell Allan and Hazard, basing on molecular analyses, as well as on profound studies on biology, morphology and lifecycle of this unique microsporidium (Sokolova and Fuxa, 2008). Unlike other microsporidia of social hymenopterans, *K. solenopsae* infects all castes and stages of the host. Each of its four developmental sequences is specialized to a certain insect caste or stage and plays a particular role in the *K. solenopsae* life cycle to promote maximum success in parasite multiplication and in vertical, horizontal, intra-, and inter-colony transmissions. Four distinctive spore types are produced: diplokaryotic spores, which develop only in brood; octets of octospores within sporophorous vesicles, the most prominent spore type in adults but never occurring in brood; Nosema-like diplokaryotic spores developing in adults; and megaspores, which occur occasionally in larvae-4 and adults of all castes but predominantly infect gonads of alates and germinate in inseminated ovaries of queens. Nosema-like spores function in autoinfection of adipocytes. Increased proliferation of diplokaryotic meronts in some cells is followed by karyogamy of diplokaria counterparts and meiosis, thereby switching the diplokaryotic sequence to octospore or megaspore development. Megaspores transmit the pathogen transovarially to the next generation. From the egg to larvae-4, infection is unapparent and can be detected only by PCR. Juvenile and megaspore sequences are abruptly triggered in larvae-4, which is the key stage in intra-colony food distribution via tropholaxis. Larvae-4 lack buccal filters, can consume solid food, and participate in horizontal transmission of spores, presumably via cannibalism and/or meconium utilization. Such adaptations, finely tuned to parasitizing fire ant colonies, make *K. solenopsae* a true social parasite, with no comparable examples among microsporidia.

Symposium - Thursday, 09:36 135

Microsporidia of lady beetles used for biological pest control.

Taro Saito - Saint Mary's University, Halifax, NS, Canada; ***Susan Bjornson*** - Saint Mary's University, Halifax, NS, Canada
Address for correspondence: susan.bjornson@smu.ca

The collection and redistribution of convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, for augmentative biological control is a practice that has persisted for almost 100 years. Beetles collected annually from their over-wintering sites in California are sold to growers and home gardeners for aphid control throughout North America. The tradition of using *H. convergens* for biological control has continued despite reports that field-collected beetles host microsporidia and other natural enemies. A microsporidium from *Hippodamia convergens* causes prolonged larval

development and reduced fecundity and adult longevity. This pathogen bears similarity to *Nosema hippodamiae*, described in the same host by Lipa and Steinhaus in 1959. The microsporidium was transmitted horizontally under laboratory conditions to four non-target coccinellids: the two-spotted lady beetle (*Adalia bipunctata*), sevenspotted lady beetle (*Coccinella septempunctata*), three-banded lady beetle (*C. trifasciata perplexa*) and the multi-coloured Asian ladybeetle (*Harmonia axyridis*). Mean spore counts from smear preparations of infected beetles suggest that the infection was as heavy in *A. bipunctata* (a native coccinellid that is also used for biological control) and *C. trifasciata perplexa* (a second native species) as it was in *H. convergens* (the natural host). Infection was lighter in *C. septempunctata* and *H. axyridis* (introduced species). The broad host range of the microsporidium in *H. convergens* raises questions regarding the identity of microsporidian pathogens from past studies of coccinellids, the impact of microsporidia on biological pest control and the role of microsporidia in regulating native beetle populations.

CONTRIBUTED PAPERS Thursday, 08:00 - 10:00
Kokopelli Ballroom III

Virus IV

Moderators: Just Vlak, Lorena Passarelli

Contributed Paper - Thursday, 08:00 136

Identification of proteins associated with *Autographa californica* nucleopolyhedrovirus budded virions.

Ranran Wang - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Fei Deng*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R,China; ***Lin Guo*** - Wuhan University, Wuhan, Hubei, P.R. China; ***Hualin Wang*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Zhihong Hu*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China
Address for correspondence: df@wh.iov.cn

The budded virion (BV) of baculovirus is specially adapted for secondary infection, spreading the virus between cells and tissues in permissive hosts. In this study, the protein composition of the BV of *Autographa californica* nucleopolyhedrovirus (AcMNPV), the type species of baculoviruses, was analysed. Using a combination of peptide mass fingerprinting and tandem mass spectrometry techniques for peptide sequencing, twenty three viral proteins associated with AcMNPV BV were identified, including PTP, P78/83, F-protein, V-ubiquitin, PP31, VP1054, ChaB-like, Ac66, IAP-2, Ac73, VLF-1, VP39, BV/ODV-E25, P6.9, BV/ODV-C42, VP80, Ac109, Ac114, GP64, Ac132, ME53, 49K and BV/ODV-EC27. Nine of these proteins, PP31, ChaB-like, Ac66, IAP-2, Ac73, Ac109, Ac114, Ac132 and ME53 were identified for the first time to be BV associated proteins. In addition, 13 host proteins were also revealed to be associated with the AcMNPV BV. A summary of the so far identified BV associated proteins was generated and the importance of these proteins in the baculoviral infection is discussed.

Contributed Paper - Thursday, 08:15 137-STU

ACMNPV *me53* is a non- essential gene required for efficient budded virus production.

Jondavid de Jong - University of Guelph, Guelph, ON, Canada; ***Basil Arif*** - Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada; ***David Theilmann*** - Pacific Agri-food Research Centre, Summerland, BC, Canada; ***Peter Krell*** - University of Guelph, Guelph, ON, Canada
Address for correspondence: jdejong@uoguelph.ca

Me53 encodes a major early transcript for a ME53 protein with a zinc-finger domain. It is a highly conserved baculovirus gene found in genomes of all lepidopteran baculoviruses that have been fully sequenced to date. We successfully developed a *me53*-null bacmid (Acme53GFP) as well as a repair virus (AcGFPRepHA) carrying *me53* with an N-terminal HA tag, under the control of its native early and late promoter elements, *Sf*9 cells transfected with Ac?me53GFP demonstrated a 3 order magnitude reduction in budded virus production when compared to both the repair and wild-type bacmids. Our data also suggests the *me53* is not involved in DNA replication and that the majority of ME53 protein is found late in the infection cycle (18-36 h.p.i.). Site directed mutagenesis of both the early and late promoter elements revealed that deletion of the early promoter resulted in a 12.5 fold reduction of budded virus production, however deletion of the late promoter resulted in a 1000-fold reduction, suggesting that, in the context of budded virus production, the predominate role of ME53 is late in the infection cycle. By Western blot ME53 was found associated with both budded and occluded derived virions. Immunofluorescence microscopy localized ME53 to both the nucleus and cytoplasm throughout infection and ME53 co-localized with p39 and gp67 at late times in the infection cycle. Together these results indicate that *me53* is not required for viral replication, however me53 plays a role in efficient budded virus production.

Contributed Paper - Thursday, 08:30 138

Majority of the f proteins from granuloviruses are functional analogues of *gp64* of ACMNPV.

Feifei Yin - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Manli Wang*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Fei Deng*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Zhihong Hu*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Hualin Wang*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China
Address for correspondence: h.wang@wh.iov.cn

The F-protein of Agrotis segetum GV (AgseGV) was identified as the first functional GV analogue of GP64 of Autographa californica nucleopolyhedrovirus (AcMNPV). In this study, GP64 of AcMNPV was substituted with F proteins from various GVs. The F protein genes of GVs were cloned and used to rescue the infectivity of gp64-null AcMNPV. It was shown that the majority of F proteins were functional analogues of GP64 of AcMNPV. However the ability of GV F proteins to rescue the infectivity GP64-null AcMNPV virus cannot be generalized. The F proteins from Choristoneura occidentalis granulovirus (ChocGV), Cydia pomonella granulovirus (CpGV), Xestia c-nigrum granulovirus (XcGV) and Agrotis segetum GV (AgseGV, previous study) can rescue the infectivity of gp64-null AcMNPV. However, we corroborate previous findings that the F protein from Plutella xylostella granulovirus (PlxyGV) cannot rescue the infectivity of gp64-null AcMNPV. Pseudotyped viruses with F proteins from CpGV, XcGV, AgseGV and other group II F proteins are easily propagated in Sf9 cell line. Pseudotyped virus with F protein from ChocGV produced a rather low level of infectious BV with titers in the order of 1µ103 IU/ml after several passages.

Contributed Paper - Thursday, 08:45 139

Identification of protein-protein interactions of the ODV associated proteins of HEARNPV.

Ke Peng - Wuhan Institute of Virology, CAS, Wuhan, Hubei,

P.R.China; ***Mingzhi Wu*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Fei Deng*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Hualin Wang*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China; ***Zhihong Hu*** - Wuhan Institute of Virology, CAS, Wuhan, Hubei, P.R.China
Address for correspondence: huzh@wh.iov.cn

Protein-protein interactions among components of occlusion-derived virus (ODV) of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV), a group II NPV, were analyzed. The ORFs of thirty-nine ODV structural proteins were cloned and expressed in the Gal4 yeast two-hybrid (Y2H) system. Totally, 1764 combinations were screened in the Y2H assay and 22 binary interactions were identified. These included P49-EC43, IE1-IE1, ODV-E56-38K, ODV-E56-PIF3, HA44-HA44, LEF3-LEF3, LEF3-Helicase, LEF3-AN, HA66-HA66, GP41-GP41, GP41-38K, GP41-HA90, CG30-CG30, 38K-38K, 38K-PIF3, 38K-PIF2, VP80-HA100, ODV-E66-PIF3, ODV-E66-PIF2, PIF3-PIF3, PIF3-PIF2 and P24-P24. The interactions of IE1-IE1, LEF3-LEF3, LEF3-Helicase, LEF3-AN and 38K-38K have been previously reported in *Autographa californica* MNPV (AcMNPV). The interactions related to HA44 and HA100 were further confirmed since they are the two newly identified ODV proteins of group II NPVs. The self-association of HA44 was verified with His pull-down assay and the interaction of VP80-HA100 was confirmed by co-immunoprecipitation assay. A summary of so far reported proteinprotein interactions of baculovirus is presented, which will further facilitate our understanding of the molecular mechanisms of baculovirus infection.

Contributed Paper - Thursday, 09:00 140

The core gene *ac96* of AVMNPV encodes a *per os* infectivity factor (*pif-4*).

Minggang Fang - Pacific Agri-Food Research Centre, Summerland, BC, Canada; ***Yingchao Nie*** - Plant Science, Faculty of Land and Food Systems, Vancouver, BC, Canada; ***Stephanie Harris*** - Saskatoon Research Centre, Saskatoon, Saskatchewan, Canada; ***Martin Erlandson*** - Saskatoon Research Centre, Saskatoon, Saskatchewan, Canada; ***David Theilmann*** - Pacific Agri-Food Research Centre, Summerland, BC, Canada
Address for correspondence: yingchao.nie@agr.gc.ca

Baculovirus core genes, those genes found in all currently sequenced baculoviruses, for the most part serve essential functions in the viral life cycle. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) *ac96* is a baculovirus core gene but its role in virus replication is unknown. To determine if it provides a critical functionality in the baculovirus life cycle, we used the AcMNPV bacmid system to generate an *ac96* deletion virus (vAc96KO). Titration assays and qPCR showed that the absence of *ac96* does not affect budded virus (BV) production or viral DNA replication. Western blotting and confocal immunofluorescence analysis showed that AC96 is expressed in the cytoplasm and nucleus throughout infection. Analysis of virion particles also showed that AC96 is a component of the envelope fraction of both BV and occlusion derived virus. Injection of vAc96KO BV into hemoceol killed *Trichoplusia ni* larvae as efficiently as repaired and control virus. However, feeding assays showed that vAc96KO was unable to infect *T. ni* larvae *per os* . Fluorescent microscopy revealed that midgut cells failed to be infected by vAc96KO which shows that ac96 encodes a new *per os* infectivity factor (PIF-4).

Contributed Paper - Thursday, 09:15 141-STU

On the binding and fusion of Baculovirus ODV to midgut epithelial cells: Distribution and orientation of *pif* proteins.

Ke Peng - Laboratory of Virology, Wageningen, Gelderland, The Netherlands; ***Jan W.M. van Lent*** - Laboratory of Virology, Wageningen, Gelderland, The Netherlands; ***Monique M. van Oers*** - Laboratory of Virology, Wageningen, Gelderland, The Netherlands; ***Zhihong Hu*** - Wuhan Institute of Virology, Wuhan, Hubei, P.R.China; ***Just M. Vlak*** - Laboratory of Virology, Wageningen, Gelderland, The Netherlands
Address for correspondence: ke.peng@wur.nl

The initial steps of infection for many enveloped viruses are binding to and fusion with cellular membranes. These processes are carried out in a concerted fashion in a complex of several viral proteins, together called the virus binding/fusion complex. Baculovirus virions exist as two phenotypes: the occlusion derived virus (ODV) and the budded virus (BV). ODVs are occluded into polyhedral occlusion bodies and are responsible for the initial infection of insect midgut epithelial cells. ODVs contain more than 10 different envelope proteins, including the four *per os* infectivity factors PIF1, PIF2, PIF3 and P74, which are essential for infectivity *per os.* PIF1, PIF2 and P74 function in virus binding while the function of PIF3 is still unknown. Up until now no fusion protein had been identified for ODV although they are believed to enter the microvillus through direct membrane fusion. P74 is C-terminally anchored in the ODV envelope with its N terminal part outside of the particle. We have employed a combination of immuno-electron microscopy and protein chemistry techniques to study the location, distribution and orientation of PIF1, PIF2 and PIF3 proteins on the ODV particles, using Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) as a experimental model. We could show that PIF1 and PIF2 proteins are present on the outside of the ODV particle and are uniformly distributed along the ODV envelope. Using in vivo crosslinking and mass spectrometry we have searched for viral and host proteins, which interact with PIF proteins. Finally, we will report on the possible role of PIF protein cleavage in the initial stage of ODV infection of midgut epithelial cells.

Contributed Paper - Thursday, 09:30 142

Fuctions of *cis*-elements of *Helicoverpa armigera* nucleopolyhedroviruses *p13* gene in early expression modulation.

Yipeng Qi - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Songya LV*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Yingle Liu*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Nan Lu*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China; ***Enqi Du*** - State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, Hubei, China
Address for correspondence: qiyipeng@whu.edu.cn

The *p13* gene is a group II baculovirus specific gene that modulate the production of the two type virions (occlusive derived virions and budded virions) of *Autographa californica* multiple nudeopolyhedrovirus. The promoter analysis of *Helicoverpa armigera* nucleopolyhedroviruses *p13* gene (Hearp13) indicated that both the early promoter motif CAT/AT and the late promoter motif TTAAG exist together with upstream homologous region hr4, and there are 2 mini upstream ORFs (uORF) containing rare codons in the 5'UTR region. Our study proved that the

Hearp13 promoter actually triggered early and late expression of the gene, the upstream hr4 sequence strongly enhanced for the expression. Using the dual Luciferase reporter system, both of the mini uORFs were demonstrated to be expressed. When the start codons of the 2 mini uORFs were mutated (i.e. ATG mutated to AAG) to prevent their translation, the expression of the downstream report gene was increased. The results mean that the mini uORFs are negative regulate elements to the expression of their downstream gene. When the rare codons in uORF sequences were substituted with the common codons to speed up the translation of the uORFs, the expression of the downstream report gene was also improved. The results mean that the mini uORFs works negatively to their downstream gene expression through the usage of rare codons.

Contributed Paper - Thursday, 09:45 143-STU

A novel gene *(orf74)* found in the *Maruca vitrata* nucleopolyhedrovirus.

Shih-Chia Yeh - Entomology, National Taiwan University, Taipei, Taiwan, Taiwan; ***Chih-Yu Wu*** - Entomology, National Taiwan University, Taipei, Taiwan, Taiwan; ***Chu-Fang Lo*** - Zoology, National Taiwan University, Taipei, Taiwan, Taiwan; ***Chung- Hsiung Wang*** - Zoology, National Taiwan University, Taipei, Taiwan, Taiwan
Address for correspondence: f94632001@ntu.edu.tw

MaviNPV, which was isolated from the infected *Maruca vitrata* larvae, is a small lepidopteran NPV. Its size is 111,953 bp and consists of 126 open reading frames (ORF) only. MaviNPV has 125 homologous genes found in *Autographa californica* multiple NPV (AcMNPV), except *orf74*. However, *orf74* is homologous to *orf34 orf98*, *orf28*, and *orf55* in *Chrysodeixis chalcites* NPV, *Orgyia pseudotsugata* MNPV, *Mamestra configurata* NPV-A, and *Leucania seperata* NPV, respectively. Whereas the identities in the amino acid sequences of these viral genes are only around 21 to 26%. The main purpose of this study is to analyze the role of *orf74* in MaviNPV, because a few homologous genes of *orf74* in GenBank are found and the function of ORF74 is still unknown. The *orf74* consists of 675 base pairs, and encodes a protein with 224 amino acids. The 5' and 3' untranslated regions (5' and 3'UTR) contain 61 and 27 base pairs, respectively. The transcription of *orf74* was first detected at 2 hours postinfection (hpi) and the highest amount at 72 hpi. The ORF74 was detected at 48 hpi and the highest amount at 72 hpi with the prepared ORF74 antibody. The location of ORF74 in the infected cells were both found in cytoplasm, around the nuclear membrane, and on the occlusion bodies with the indirect fluorescent immunoassay and immunogold. The function and the interaction with other proteins of ORF74 need to be confirmed in the future.

10:30 - 12:00 - SIP Annual Business Meeting
Kokopelli Ballroom II-III

Retired SIP Members: Where are our old friends now?

Elizabeth W. Davidson - Arizona State University, Tempe, AZ, USA
Address for correspondence: e.davidson@asu.edu

Many of our colleagues who were very important in establishing and managing SIP and invertebrate pathology in its early years have retired and no longer attend our meetings. We have contacted several of them and will bring the current members up to date on their activities and a bit about why they were very important to the Society. We owe them a lot!

Symposium (Virus Division) Thursday, 13:30 - 15:30
Kokopelli Ballroom III

The Viral Face of PDV's: Origin and Structure of the Chromosomally Integrated PDV Genomes

Organizers/Moderators: Jean-Michel Drezen, Sassan Asgari

Symposium - Thursday, 13:30 144

Phylogenomic approaches unravel the origin and tempo of bracovirus evolution.

Elisabeth Herniou - IRBI - UMR CNRS 6035, Tours, Indre et Loire, France; ***Julien Theze*** - IRBI - UMR CNRS 6035, Tours, Indre et Loire, France; ***Annie Bezier*** - IRBI - UMR CNRS 6035, Tours, Indre et Loire, France; ***Georges Periquet*** - IRBI - UMR CNRS 6035, Tours, Indre et Loire, France; ***Jean-Michel Drezen*** - IRBI - UMR CNRS 6035, Tours, Indre et Loire, France
Address for correspondence: elisabeth.herniou@univ-tours.fr

Recent genomic data revealed the nudiviral origin of the polydnaviruses of braconid wasps. Here we assembled all the viral genomic data available for bracoviruses to perform detailed phylogenetic analyses of the relationships between bracoviruses, nudiviruses, baculoviruses and salivary gland hypertrophy viruses. We performed multiple alignements of the 19 bracovirus genes of viral origin. We verified the phylogenetic signal congruence between all the genes, before concatenating the alignements for the phylogenetic reconstruction of the evolutionary history of bracoviruses. The tree showed that nudiviruses are paraphyletic with the inclusion of bracoviruses within the clade. Bracoviruses indeed derive from nudiviruses. The analyses of evolutionary rates revealed that the transition from free living to obligatory symbiotic virus has led to changes in the selection pressure put upon the viral genes. Lastly, since this phylogeny is connecting virus with braconid wasp evolution, we were able to use fossil data to date the virus tree.

Symposium - Thursday, 14:00 145

Genetic changes in bracoviruses associated with host shifts in braconid parasitoid wasps.

James Whitfield - University of Illinois, Urbana, Illinois, USA; ***Michael Strand*** - University of Georgia, Athens, Georgia, USA
Address for correspondence: jwhitfie@life.uiuc.edu

Bracoviruses associated with microgastroid parasitoid wasps play a major role in successful parasitization of host caterpillars by the wasps. Recent genomic/pyrosequencing breakthroughs allow us to obtain bracovirus genome sequences virtually in their entirety from individual wasp individuals, opening up the possibility of studying on a broader scale the actual genetic changes in bracoviruses between related wasps that now specialize in parasitizing different hosts. A massive inventory survey of caterpillars and their associated parasitoid wasps in Costa Rica now provides an opportunity to make such genomic comparisons and a large scale within certain microgastrine wasp genera. We discuss strategies for comparing the bracovirus genomes and identifying genes under differential selection in different hosts.

Symposium - Thursday, 14:30 146

Chromosomally integrated glyptapanteles bracovirus genomes: Structure and organization

Dawn Gundersen-Rindal - USDA ARS Invasive Insect Biocontrol & Behavior Laboratory, Beltsville, MD, USA; ***Chris Desjardins*** - J. Craig

Venter Institute, Rockville, MD, USA; ***Vish Nene*** - J. Craig Venter Institute, Rockville, MD, USA
Address for correspondence: dawn.gundersen-rindal@ars.usda.gov

Glyptapanteles indiensis and *G. flavicoxis* (Braconidae, Hymenoptera) parasitoid wasps possess obligate endosymbiont polydnaviruses essential for successful parasitization of larvae of the lepidopteran *Lymantria dispar* (gypsy moth) pest. In both these species, 29 variable sized circular dsDNA segments are encapsidated into infectious bracovirus particles in a process dependent upon the linear provirus form of the virus integrated chromosomally within the parasitoid wasp. Bracovirus particles are injected into larval hosts during oviposition where virus-associated immunosuppression and other negative impacts within the larval host ensure survival of the parasitoid and, thus, the provirus form. Recent analyses of the two *Glyptapanteles* parasitoid species have characterized genome sequences and organization for both the encapsidated virus form, which lacks genes for structural proteins, and the chromosomally integrated provirus form. The encapsidated *Glyptapanteles* viral genomes encode numerous genes conserved among the bracoviruses characterized to date as well as novel genes that may originate from the parasitoid. The proviral segments in both *Glyptapanteles* species are organized as multiple loci containing one to many viral segments that are separated from each other by flanking parasitoid gene-encoding chromosomal DNA and share other features for discussion.

Symposium - Thursday, 15:00 147

A viral origin for ichnovirus particles?

Anne-Nathalie Volkoff - INRA, Montpellier, France; ***Jean-Michel Drezen*** - CNRS, Tours, France; ***Véronique Jouan*** - INRA, Montpellier, France; ***Serge Urbach*** - Plate-forme Protéomique CNRS UMR 5203, INSERM U661,UM1, UM2, Montpellier, France; ***Gabor Gyapay*** - CEA-GENOSCOPE-Centre National de Séquençage, Evry, France
Address for correspondence: volkoff@supagro.inra.fr

A viral origin of polydnaviruses could not be established until very recently with the demonstration by Bezier *et al.* (2009) that bracoviruses originate from an ancestral association between a wasp and a nudivirus. Concurrently, no nudiviral genes were found for ichnoviruses (IVs), suggesting that viral machineries differ between these two polydnavirus genera. The viral origin of modern IVs thus remains unsolved. To explore this question, we focused on genes involved in morphogenesis of the IV associated with *Hyposoter didymator* (HdIV). We searched for HdIV structural proteins by a mass spectrometry approach and investigated the wasp genomic environment of homologs of CsIV *p12* and *p53* genes. Altogether, we were able to identify more than 25 proteins associated to HdIV virions. Interestingly, our results indicate that genes encoding components of HdIV particles are clustered within "specialized" genomic regions of the associated wasp host. These regions could represent fingerprints of still non-identified ancestral viral sequences.

Symposium (Nematode Division) Thursday,13:30 - 15:30
Painted Horse I-II

Ecological Interactions in Entomopathogenic Nematodes

Organizers/Moderators: Albrecht Koppenhofer, Harry Kaya

Symposium - Thursday, 13:30 148

Risk-sensitive infection strategies: A new way to look at parasite behavior.

Edwin Lewis - Department of Nematology, Davis, CA, USA; ***James Campbell*** - USDA, Manhattan, KS, USA; ***Hsieh Fushing*** - Department of Statistics, Davis, CA, USA; ***David Shapiro-Ilan*** - USDA, Byron, GA, USA; ***Glen Stevens*** - Ferrum College, Ferrum, VA, USA
Address for correspondence: eelewis@ucdavis.edu

We offer a new and useful theoretical framework in which to study the infection behavior of parasites. Infection behavior can be broken down into a series of 'decisions' made by a foraging parasite, each of which is a tradeoff between risk and reward modulated by an animal's internal status (nutritional needs, energy reserves, etc.) and its surrounding environment. The infective juvenile stage of entomopathogenic nematodes can discriminate among potential host species based upon their perceptions of host quality and suitability. In prior research, the emphasis has been on measurements of host recognition and suitability for uninfected hosts, and the parasite's decision of whether or not to initiate an infection. But in this system where tens to hundreds of infective juveniles invade a single host, the overwhelming majority join an ongoing infection. We use a group-event history model to explain the group dynamics of entomopathogenic nematodes when confronted with the decision of whether or not to invade a host of varying quality. We borrow the theoretical construct of "herding", as developed in the fields of economics and finance, to help describe the group behaviors involved in entomopathogenic nematode infections. Mathematically and behaviorally, infection behavior of entomopathogenic nematodes resembles buying activity on Wall Street. Experimentally, we evaluated the determinates of infection and show that for short periods of time, infective juveniles actively prefer hosts that are already infected, just as stock brokers buy stocks that are already being bought at a high rate. Thus the infection status of a host has profound effects on infective juvenile behavior towards hosts. This study is the first we know of that examines the host finding and infection behaviors of parasites in the context of risk sensitivity.

Symposium - Thursday, 13:54 149

Virulence and infectivity of entomopathogenic nematodes: Changes with age.

Christine T. Griffin - National University of Ireland Mayooth, Maynooth, County Kildare, Ireland; ***Adam G. Guy*** - National University of Ireland Mayooth, County Kildare, Ireland; ***Denis J. Wright*** - Imperial College London, Berkshire, UK; ***Michael T. Gaffney*** - Teagasc, Dublin, Ireland
Address for correspondence: christine.griffin@nuim.ie

Steinernema and *Heterorhabditis* develop in killed insect hosts and typically emerge from the host cadaver as infective juveniles. These juveniles are rich in lipid reserves which provide fuel for the juveniles' host-seeking and survival in soil. As their reserves are depleted, the ability of the juveniles to infect and kill insects declines and they eventually die. However, performance does not necessarily decline at a constant rate, and may even increase initially before declining. We will review earlier findings, including that *Heterorhabditis megidis* infective juveniles became more infective when tested some weeks after emergence from the natal cadaver, and present new data which show that similar effects occur in other species of entomopathogenic nematode (*Heterorhabditis downesi, Steinernema carpocapsae* and *Steinernema kraussei*). The ecological and applied significance of changes in infectivity and virulence will be discussed.

Symposium - Thursday, 14:18 150

Overcoming antagonists and environmental hazards.

Claudia Dolinski - UENF/CCTA/LEF, Campos dos Goytacazes, RJ, Brazil; ***David Shapiro-Ilan*** - USDA-ARS, SEFTNRL, Byron, GA, USA
Address for correspondence: claudia.dolinski@censanet.com.br

Entomopathogenic nematode survival is fraught with numerous biotic and abiotic challenges. Biotic antagonists include a variety of organisms such as pathogens (e.g., phages and protozoa), nematophagous fungi, and arthropod predators (e.g., mites and Collembola). Infected host cadavers containing entomopathogenic nematodes may also be attacked by scavengers, though mechanisms to deter certain scavengers have evolved in heterorhabditids species. Additionally, entomopathogenic nematode survival can be suppressed in a more indirect fashion through competition. Furthermore, entomopathogenic nematode populations can be severely limited by environmental factors such as desiccation, temperature extremes, and oxygen stress. For example, the nematode's susceptibility to ultraviolet radiation and desiccation continues to discourage aboveground applications. Interactions with chemicals in the environment can also impact nematode survival. Chemical agents may include various pesticides and fertilizers. Biotic and abiotic factors that negatively affect nematode survival lead to a reduction in biocontrol potential. On the other hand, positive interactions between entomopathogenic nematodes and soil biota or chemicals are also possible. In this presentation, we discuss our current understanding of factors affecting nematode survival and fitness, and introduce recent innovations for overcoming adversarial conditions and enhancing biocontrol efficacy. Innovations include strain selection, improved application methods, novel formulations, and habitat manipulation.

Symposium - Thursday, 14:42 151

Spatio-temporal nematode-host interactions in turfgrass.

Albrecht Koppenhöfer - Dept. Entomology, Rutgers University, New Brunswick, NJ, USA; ***Benjamin McGraw*** - Dept. Golf and Plant Sciences, State Univeristy of New York, Delhi, NY, USA
Address for correspondence: koppenhofer@aesop.rutgers.edu

Long-term effects of recycling entomopathogenic nematode (EPN) populations are one of the advantages they have over synthetic insecticides for pest management. A good understanding of interactions between populations of EPN and their hosts in space and time is therefore paramount in optimizing and increasing the use of these biocontrol agents. Turfgrass in one of the better studied systems with respect to spatial and/or temporal interactions of natural and released EPN populations and their hosts. Klein and Georgis (1992) observed suppression of *Popillia japonica* larval populations by inundatively released *Heterorhabditis bacteriophora* into the following host generation. Campbell et al. (1995) found that *P. japonica* larval densities were about 50% lower in host patches that overlapped with endemic *H. bacteriophora*, and inundatively released *H. bacteriophora* quickly returned to a patchy distribution similar to that of endemic *H. bacteriophora* (Campbell et al. 1998). Koppenhöfer and Fuzy (2009) studied the effect of a wide range of inoculative application of the scarab-specific *Steinernema scarabaei* on *Anomala orientalis* larval populations. *S. scarabaei* suppressed *A. orientalis* for at least 2 years after releases, irrespective of application rate, and persisted for up to 4 years in the experimental plots. *S. scarabaei* densities had a significant negative correlation with *A. orientalis* densities. *S. scarabaei* co-existed with endemic *H. bacteriophora* and *S. carpocapsae*. McGraw and Koppenhöfer (2009) studied spatio-temporal interactions between endemic *H. bacteriophora* and *S. carpocapsae* and *Listronotus maculicollis* on golf course fairways. Both EPN species infected all *L. maculicollis* stages between third instar and teneral adult and demonstrated a distinct seasonality coinciding with the peak in *L. maculicollis* soil stage densities. But *L. maculicollis* generational mortality due to EPN infection was highly variable (0-50%) between host generations and years, and EPN and *L. maculicollis* spatial dispersion were not significantly related.

Symposium - Thursday, 15:06 152

Ecological dynamics of entomopathogenic nematodes in a natural system.

Daniel Gruner - University of Maryland, College Park, MD, USA; ***Donald Strong*** - University of California - Davis, Davis, CA, USA
Address for correspondence: dsgruner@umd.edu

Entomopathogenic nematodes (EPN) are increasingly employed as biological control agents for root-feeding or soil-dwelling insects in managed systems. However, most practitioners use inoculative or augmentative practices, and there are few documented cases of persistent classical biological control. In natural populations in a California coastal prairie, persistent populations of *Heterorhabditis marelatus* have been studied continuously for 15 years. An ecologically important host insect in this habitat, the larvae of the hepialid lepidopteran *Hepialus californicus*, attacks, girdles and kills the dominant woody shrub, the yellow bush lupine (Fabaceae: *Lupinus arboreus*). Field and laboratory experiments show that *Heterorhabditis marelatus* predation on this moth species indirectly benefits *Lupinus arboreus* through a top-down trophic cascade. Multi-year, spatially explicit studies demonstrate that the strength of these interactions varies temporally and spatially, and caterpillar outbreaks and lupine devastation can occur even within a background of robust EPN populations. Desiccation risk during Mediterranean summers drove EPN into localized extinction or into moisture refugia, both within lupine rhizospheres and into deep (>80 cm) soils lacking host insects. Monthly samples of soil columns from grassland and lupine rhizospheres over one year confirm that lupine canopies facilitate EPN populations with higher soil moisture and by harboring greater diversity and abundance of arthropod larvae in the soil. These features create greater opportunity under lupine canopies for localized individual movement, host-finding behavior, and survival of IJs, and promote recycling of additional EPN generations through *Hepialus* as well as alternative arthropod hosts. However, limited locomotion – both by nematodes and insect hosts in soil – impair EPN aggregative recruitment to high local host densities and reinforce the inherent patchiness of naturally-occurring EPN populations. Metapopulation dynamics, which can override limitations set by local and short-term abiotic conditions, may ultimately determine long-term EPN persistence in this natural system.

CONTRIBUTED PAPERS Thursday, 13:30 - 14:30
White Pine I-II

Microbial Control III

Moderator: Jean Maniania

Contributed Paper - Thursday, 13:30 153

Evaluation of *Iisaria fumosorosea* to control the Asian citrus psyllid, *Diaphorina citri*.

Steven Arthurs - Mid-Florida Research and Education Center, Apopka, FL, USA; ***Pasco Avery*** - Indian River Research and Education Center, Fort Pierce, FL, USA
Address for correspondence: spa@ufl.edu

Its introduction in Florida in 1998, the Asian citrus psyllid, *Diaphorina*

citri Kuwayama, has become a serious concern for citrus growers throughout the state. However, the pest status of *D. citri* has increased rapidly following the confirmation of citrus greening disease in Florida 2005. The ability of this phloem feeding insect to spread the bacteria to new groves has resulted in a large increase in the amounts of chemical insecticides used to control this pest. The use of mycoinsecticides to against *D. citri* is attractive due to limited pre-harvest and restricted entry intervals and compatibility with IPM approaches for concurrent citrus pests. Currently several strains of *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*) are promising candidates for control of *D. citri*. We will present data evaluating several isolates of *I. fumosorosea* for activity against *D. citri*. Dose response tests were conducted with a commercial blastospore formulation (PFR-97 WDG) and conidial formulation of Apopka 97 strain (CX 2105), along with additional isolates ARSEF 3581 and FE9901. The most promising strains will be used in scaled up studies for season long control of *D. citri*.

Contributed Paper - Thursday, 13:45 154

Efficacy of fungal pathogens as biologically-based agents for control of adult mosquitoes.

Nancy E. Beckage - University of California-Riverside, Riverside, CA, USA; ***Anita R. Gordillo*** - University of California-Riverside, Riverside, CA, USA
Address for correspondence: nancy.beckage@ucr.edu

Mosquitoes are continuing to evolve high levels of pesticide resistance worldwide, invoking re-introduction of DDT and other toxic chemical insecticides as adulticides in some areas of Africa and elsewhere to reduce rates of vector-borne disease transmission. Recent refocusing of efforts on development of inexpensive, sustainable, and effective biologically-based biopesticides has generated a resurgence of interest in the use of fungal pathogens as adulticides to control adult culicine as well as anopheline vectors to reduce rates of transmission of malaria, dengue, filiariasis, and other parasites and pathogens. Different methods of fungal dissemination and infection of *Culex* sp. by *Metarhizium anisopliae* were tested. Results will be presented in the context of ongoing studies of *Beauvaria bassiana* as well *Metarhizium anisoplieae* taking place in both laboratory and field settings around the world. Prospects for future implementation of fungal pathogens as biological control agents at field sites for adult mosquito control in villages in Mali, West Africa, will be presented.

Contributed Paper - Thursday, 14:00 155

Evaluation of entomopathogenic fungi for the management of *Sternochetus mangiferae* on mango.

Sunday Ekesi - International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Nairobi, Kenya; ***Rabiu Salisu Adamu*** - Ahmadu Bello University, Zaria, Kaduna, Nigeria; ***Nguya Kalemba Maniania*** - International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Nairobi, Kenya
Address for correspondence: sekesi@icipe.org

The mango seed weevil (MSW) *Sternochetus mangiferae* (F.) (Coleoptera: Curculionidae) is a major pest of mango in Kenya causing damage that ranged between 30-72% depending on the cultivar, locality and season. Quarantine restrictions on weevil-infested mango fruits restrict export to large lucrative markets in Europe, the Middle East, Japan and USA, where the insect is a quarantine pest. In this study, we evaluated 17 isolates of *Metarhizium anisopliae* and *Beauveria bassiana* for their pathogenicity to adult weevil. All isolates tested were pathogenic to the weevil incurring mortality of 15-94% at 14 days post treatment. In the dose response relationship study, using the highly pathogenic isolates (*M. anisopliae* ICIPE 62, 69 and 2 Bug), the lethal concentration causing 50% mortality (LC_{50}) in MSW was found to be lower in insects treated with ICIPE 62 ($8.4 \times 10^4 \pm 0.3 \times 10^4$) followed by ICIPE 69 and 2 Bug ($6.4 \times 10^5 \pm 0.2 \times 10^4$; $2.5 \times 10^6 \pm 0.1 \times 10^6$, respectively). The conidial persistence of oil formulation of isolate ICIPE 62 applied to mango trunk was longer (> 3 weeks) compared with the aqueous formulation (2 weeks). Conidial persistence was not affected by mango cultivar. In field cage test, topical application of oil formulation of isolates ICIPE 62 on weevils hibernating on mango trunk resulted in 92% mortality of the weevil after 12 days post treatment compared with 86% mortality in residual application to the trunk. The significance of the treatment application with regard to weevil hibernation and dispersal will be discussed.

Contributed Paper - Thursday, 14:15 156

Efficacy of *Bacillus thuringiensis* var. *israelensis* strain ABG-6193 against field-collected larvae of three Culicine mosquito species.

Samy Hussein Mohamed - Plant Protection Dept, Assiut, Assiut, Egypt
Address for correspondence: samy_hussein@yahoo.com

Six concentrations of the pathogenic preparation ABG-6193 (*Bacillus thuringiensis var. israelensis*) were tested to estimate its larvicidal activity against the fourth instar field collected larvae of *Culex pipiens molestus* Forskal, *Culex antannatus* Becker and *Aedes caspus* Pallas in the laboratory. Results revealed that all the tested concentrations of the pathogenic preparation were effective against the tested culicine mosquito species, and mortality increased as the concentration and exposure period increased. The greatest concentration tested of 32.23 ppm was more active against the fourth instar larvae of all tested mosquito than the other ones, and according to mortality and exposure period. At this concentration the LT_{50} values were 0.553, 0.248 and 0.496 hours for the forward three mosquito species, respectively. Whereas, the lowest concentration of 1.25 ppm was the least effective one and it exhibited LT_{50} of 1.672, 0.662 and 0.886 hours, respectively. The susceptibility differed among the three mosquito species; *Culex antannatus* was the most susceptible species to the six tested concentrations, whereas *Culex pipiens molestus* was the least one.

Contributed Paper - Thursday, 14:30 157

Possible use of Metarhizium for controlling the lesser mealworm, Alphitobius diaperinus in broiler houses.

Galina Gindin - The Volcani Center, Beit Dagan, Israel; ***Asayel Rot*** - Kimron Veterinary Institute, Beit Dagan, Israel; ***Avishai Lublin*** - Kimron Veterinary Institute, Beit Dagan, Israel; ***Itamar Glazer*** - The Volcani Center, Beit Dagan, Israel; ***Michael Samish*** - Kimron Veterinary Institute, Beit Dagan, Israel
Address for correspondence: samishm@int.gov.il

The lesser mealworm, Alphitobius diaperinus, is a cosmopolitan pest of poultry and poultry houses. The last-instar larvae tunnels through soft materials while looking for pupation sites as wellas and serves as reservoirs of avian and human pathogens. The beetles' eggs and larvae develop on/in the litter. This pest control is highly challenging due to their propensity to hide and the limitations on using chemicals in poultry houses. We tested the pathogenicity of 18 Metarhizium anisopliae (Metschn.) Sorokin isolates and 22 Beauveria bassiana (Balsamo) Vuillemin isolates against Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) larvae and adults. The efficacy of the most virulent isolate

– M. anisopliae (K – was evaluated in containers (18 µ 18 µ 11 cm) with a concrete bottom covered with wood shavings, under simulated poultry house conditions. Application of conidia of this isolate to the shavings or directly to the concrete bottom reduced the yield of larvae by 8-15 times compared with the control. In another test, the mortality of mature larvae placed on previously inoculated shavings or bottom reached 80-90% within 14 days, compared with 14% in the control. The residual activity of conidia kept at 28°C retained its initial level during 14 days post-inoculation, but declined after 3 weeks. In a mini field experiment (1.0x.02x0.7m containers, 35 chicks/container), the amount of larvae found in the fungi-sprayed containers shortly after introducing 1d old broiler chicks was lower by 98.6% in comparison to the control. Dipping 1d old commercial broiler chicks in conidia suspension (1X108 /ml) did not affect their health and/or their development. We conclude that M. anisopliae has considerable potential for the control of A. diaperinus in broiler houses if the conidia will be applied on clean ground shortly before restocking with new birds.

Contributed Paper - Thursday, 14:45 158

The effects of a granulovirus infection on the growth and development of *Helicoverpa armigera* larvae.

Gustav Bouwer - School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, Gauteng, South Africa; ***Garry Coulson*** - School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, Gauteng, South Africa
Address for correspondence: gustav.bouwer@wits.ac.za

The African cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a serious pest of many agricultural crops and causes economic losses all over the world. As part of the evaluation of baculoviruses as control agents of *H. armigera* in South Africa, the effect of a *H. armigera* granulovirus (HearGV) infection on the growth and development of *H. armigera* was evaluated. After determining the volume imbibed by neonate larvae, bioassays were performed on neonate larvae using the droplet feeding method and gradient-purified occlusion bodies. The median lethal dose (LD_{50}) and median survival time (ST_{50}) of HearGV were determined. Since HearGV had a slow speed of kill, the study provided support for the classification of HearGV as a Type 1 GV. HearGV infection increased the duration of some larval instars and the larval period of infected insects was up to 8 days longer than that of the controls. HearGV had a pronounced effect on larval weight gain, with HearGVinfected larvae gaining weight at a slower rate than uninfected controls. The maximum weight of HearGV-infected larvae was heavier than that of the controls. LD_{90} -inoculated larvae were heavier than LD_{50} -inoculated larvae, suggesting that there may a relationship between larval weight and HearGV dose.

Contributed Paper - Thursday, 15:00 159

Methods for testing side-effects of pesticides on *Neozygites floridana.*

Vitalis Wekesa - Departament of Entomology, Plant Pathology and Agricultural Zoology, Escola Superior de Agricultural, Piracicaba, Sao Paulo, Brazil; ***Markus Knapp*** - Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands; ***Italo Delalibera*** - Departament of Entomology, Plant Pathology and Agricultural Zoology, Escola Superior de Agricultural, Piracicaba, Sao Paulo, Brazil
Address for correspondence: ewekesah@yahoo.com

The objective of this work was to test the effect of selected pesticides used in tomato production on different stages of the life cycle of *Neozygites floridana* Weiser & Muma, a fungal pathogen of the tomato red spider mite, *Tetranychus evansi* Baker & Pritchard. Here we describe a method where selected pesticides were tested on this fungus in two different concentrations, the mean commercial rate (CR) and 50% of the mean commercial rate (CR/2). Mummified *T. evansi* (brown fungus-killed mite cadavers) or the substrates used for sporulation (leaf discs and coverslips) were either immersed or sprayed with pesticides before testing the effects on sporulation, germination of primary conidia and infectivity of *N. floridana*. Effect of pesticides on in vivo development of *N. floridana*. was tested by feeding the *T. evansi* mites on pesticide contaminated leaf discs before infecting them with the fungus and determining their mortalities. The effects of pesticides on sporulation, germination and infectivity of *N. floridana* varied according to the chemical nature, concentration, and the method of application. Direct immersion of mummified cadavers, coverslips or leaf discs into pesticides showed stronger effects on sporulation and germination than the spray tower method, although infectivity of capilliconidia was not affected by the the method of application. The fungicides Captan and Mancozeb presented higher reduction in sporulation and germination in both concentrations. Propargite did not inhibit sporulation but affected germination of primary conidia. Methomyl and Abamectin resulted in less effects on different stages of the life cycle of *N. floridana*.

Symposium (Cross-Divisional) Thursday, 16:00 - 18:00
Kokopelli Ballroom II

Multitrophic Interactions: Implications for Invertebrate Pathogens

Organizers/Moderators: Jenny Cory, Helen Roy

Symposium - Thursday, 16:00 160

Multitrophic level interactions and population dynamics.

Saskya van Nouhuys - University of Helsinki & Cornell University, Helsinki, Finland
Address for correspondence: sdv2@cornell.edu

Species at different trophic levels interact directly, say as predator and prey. They also interact indirectly, via their shared relations with a third species. The outcomes of these indirect interactions may be complex and difficult to predict. For instance an herbivore could either benefit or suffer from feeding on a chemically defended plant, depending on response of natural enemies to the plant defense. While these multitrophic level interactions are complicated and both positive and negative for all species involved, they do not simply sum to zero. Their outcome can be observed at the scale of population dynamics. I will present two examples involving the Glanville fritillary butterfly and its parasitoid in Finland. In the first, the spatial distribution of two host plant species affects parasitoid metapopulation dynamics. In the second, a complex interaction between a plant, a phytopathogen, an herbivore and a parasitoid results in a positive association between the phytopathogen and the parasitoid at a landscape scale.

Symposium - Thursday, 16:30 161

Scared sick? Predator-pathogen facilitation strengthens herbivore suppression.

Ricardo Ramirez - Texas A&M University, College Station, TX, USA; ***William Snyder*** - Washington State University, Pullman, WA, USA
Address for correspondence: ricarrami@tamu.edu

Herbivore suppression often strengthens with increasing predator

biodiversity, but little is known about the role of entomopathogens in these relationships. Entomopathogens and predators are two classes of natural enemy that exhibit differences in ecologically important traits (e.g., size, resource acquisition strategy, foraging location) that could lead to complementary effects on shared prey/hosts. We manipulated species richness among a community of predators and entomopathogens that together attack the Colorado potato beetle, *Leptinotarsa decemlineata*, and measured resulting effects on beetle suppression and resulting plant damage. We found that beetle mortality increased, and plant damage decreased, when more natural enemy species were present. However, closer examination revealed that it was the pairing of predator with entomopathogen species, rather than greater biodiversity *per se*, that strengthened herbivore suppression. In this community predators (*Hippodamia convergens*, *Nabis alternatus*, and *Pterostichus melanarius*) occur aboveground, attacking beetle stages feeding on plant foliage, whereas entomopathogens (*Steinernema carpocapsae*, *Heterorhabditis marelatus*, and *Beauvaria bassiana*) occur belowground and attack beetles pupating in the soil. In a subsequent field experiment we tracked the emergence of predator-pathogen complementarity throughout the course of beetle development. We found that beetles exposed to predators aboveground were more susceptible to subsequent entomopathogen infection belowground, consistent with our observation in the laboratory that predator exposure weakens beetles' immune response. Thus, predators facilitated resource capture by entomopathogens, perhaps due to conflicting energetic demands for anti-predator versus anti-pathogen defenses. Our results suggest that predator-pathogen combinations were particularly taxing not because the natural enemy species partitioned resources among themselves, but instead because they enforced the partitioning of resources internal to prey/host individuals.

Symposium - Thursday, 17:00 162

Multitrophic interactions – Are entomopathogens and parasitoids good for each other?

Jenny Cory - Simon Fraser University, Burnaby, British Columbia, Canada
Address for correspondence: jsc21@sfu.ca

There is a tendency to believe that biological control agents will interact together in a positive way to enhance pest control, even though in reality they are often competing for the same resource. Numerous laboratory studies have looked at the interactions between a variety of insect pathogens and natural enemies at an individual level but the overall outcome of this competition and the implications for biocontrol in the field is not clear. Here I review the interactions between entomopathogens and one group of natural enemies, the parasitoids, to see if it is possible to gain a general picture of when these interactions are positive in terms of pest control and whether there are circumstances when they in fact compete.

Symposium - Thursday, 17:30 163

Interactions involving entomopathogenic fungi and insects: An applied perspective.

Jason Baverstock - Rothamsted Research, Harpenden, Hertfordshire, UK; ***Helen Roy*** - Centre for Ecology and Hydrology, Wallingford, Oxfordshire, UK; ***Judith Pell*** - Rothamsted Research, Harpenden, Hertfordshire, UK
Address for correspondence: jason.baverstock@bbsrc.ac.uk

The use of entomopathogenic fungi as control agents of pests has traditionally followed the principals of inundative biological control where a virulent strain is isolated, mass produced and applied to crops in the form of sprays. Whilst this has been successful for Hypocreales such as *Beauveria bassiana* and *Metarhizium anisopliae,* there has been less success in the formulation and mass production of the Entomophthorales. Despite this the host-specificity exhibited by the Entomophthorales ensures that they remain an attractive option within alternative biological control strategies. At present the efficacy of both groups of fungi within guilds of insect natural enemies is being assessed as part of conservation biological control schemes. The success of these schemes will depend on the interactions that occur between the pest and its enemies (multitrophic interactions) and those between the enemies themselves (intraguild interactions). Whereas positive intraguild interactions may benefit pest control, negative intraguild interactions may have a detrimental effect. Alternatively the avoidance of a fungal pathogen by its target insect may render it useless as a biological control agent. Here we describe interactions that occur between entomopathogenic fungi and arthropods in a range of cropping systems. The ability of arthropods to detect and respond to fungal pathogens will be discussed alongside the effect of arthropod foraging on the transmission and vectoring of fungi. Finally the manipulation of insect behavior for the auto-dissemination of fungal pathogens will be described.

CONTRIBUTED PAPERS Thursday, 16:00 - 18:00
White Pine I-II

Microbial Control IV

Moderator: Vitalis Wekesa

Contributed Paper - Thursday, 16:00 164

Broad spectrum potential of the biopesticide, *Isaria fumosorosea* for managing insect pests of citrus.

Pasco B. Avery - University of Florida, Institute of Food and Agricultural Sciences, Fort Pierce, FL, USA; ***Wayne B. Hunter*** - USDA, ARS, Ft. Pierce, FL, USA; ***David G. Hall*** - USDA, ARS, Ft. Pierce, FL, USA; ***Mark A. Jackson*** - USDA, ARS, Crop Bioprotection Research Unit, Peoria, IL, USA; ***Charles A. Powell*** - University of Florida, Institute of Food and Agricultural Sciences, Fort Pierce, FL, USA; ***Michael E. Rogers*** - University of Florida, Citrus Research and Education Center,
Address for correspondence: pbavery@ufl.edu

In Florida, growers are concerned about the ecological and economic ramifications of being dependent upon insecticide applications for the management of the Asian citrus psyllid, *Diaphorina citri*, the insect vector of the pathogen which causes Huanglongbing. In addition, there is concern about the potential resurgence of secondary pests of citrus. In collaboration with citrus growers on the East Coast, we have been evaluating approaches that would be more IPM compatible in the long term. In 2007, an entomopathogenic fungus was found infecting *D. citri* in citrus groves in Polk County as *Isaria fumosorosea* (= *Paecilomyces fumosoroseus* (*Pfr*)). Since this discovery, we have been evaluating the efficacy of different fungal strains of *Pfr* formulations (ARSEF *Pfr* 3581, *PFR* 97™ 20% WDG) as an alternative treatment. This presentation will highlight some of the results from laboratory, greenhouse and field studies completed or ongoing for assessing the efficacy of the *Pfr* formulations for managing insect pests of citrus.

Contributed Paper - Thursday, 16:15 165

Control of pine weevil *Hylobius abietis* with entomopathogenic nematodes, and safety of nematodes to nontarget insects.

Christine T. Griffin - National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland; ***Aoife B. Dillon*** - Coillte, Cork, Ireland; ***Darragh Ennis*** - National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland; ***Khalil M. Alameen*** - National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland; ***Aileen Foster*** - National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland; ***Chris D. Harvey*** - National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland
Address for correspondence: christine.griffin@nuim.ie

The large pine weevil *Hylobius abietis* is the most serious pest of reforestation in northern Europe, with a life cycle similar to that of *H. congener* in north America. Weevil larvae feed under the bark of stumps of recently felled conifers. When adult weevils emerge they feed on the bark of newly planted tree seedlings; 100% mortality of unprotected trees is not uncommon. The strategy of targetting pine weevil larvae and pupae within the stumps has potential to reduce weevil populations. In small scale field trials *Heterorhabditis downesi* was the most successful nematode species tested, reducing the emergence of adult weevils by up to 85% compared to untreated controls. *Steinernema carpocapsae* performed surprisingly well, given its reputation as an ambush forager: it parasitised weevils that were located inside tree roots up to 50 cm below soil level, and reduced emergence of adult weevils by up to 64%. Laboratory experiments indicate that host-finding by *S. carpocapsae* may be facilitated by roots as routeways through the soil, especially when there are weevil larvae feeding on them. Large scale trials of nematodes against pine weevil are underway in commercial forests in Ireland, with approximately 370 hectares treated to date. No adverse effects of nematodes on numbers or diversity of non-target beetles, either wood-associated or not, were detected by comparing trap catches from nematode-treated and untreated stumps. Special attention was paid to the non-target *Rhagium bifasciatum*, a common longhorn beetle which is important in wood decomposition. Although all stages of this beetle are susceptible, they escape nematode attack by spatial and temporal separation from the nematodes. At the time of nematode application to sites, *R. bifasciatum* is present only in debris logs which are at an advanced stage of decay, while nematodes are applied only around tree stumps where persistence is limited.

Contributed Paper - Thursday, 16:30 166

Field efficacy of *Beauveria bassiana* on the *Vespula germanica* wasp nests.

Loreto Merino - NIA Quilamapu, Chillán, Bio Bio, Chile; ***Andrés France*** - NIA Quilamapu, Chillán, Bio Bio, Chile; ***Marcos Gerding*** - NIA Quilamapu, Chillán, Bio Bio, Chile; ***Ricardo Ceballos*** - NIA Quilamapu, Chillán, Bio Bio, Chile
Address for correspondence: lmerino@inia.cl

After an extensive survey along Chile and selection process in the lab, the isolate DW B941 of *Beauveria bassiana* was selected for field experiments against *V. germanica* nest control. The first evaluation was accomplished on nest collected from the field and reduced to a population of 100 larvae, 25 workers and 1 queen, and confined into wood boxes. The wasp colonies were feeding with liquid baits containing 1 x 10^8 conidia mL^{-1} of *B. bassiana.* The mortality of larvae and workers were evaluated daily. The second experiment was similar but achieved in three fields on wild nests. The bait was changed every 5 days and the insect traffic was evaluated weekly. After 5 weeks the nests were removed from the soil and the adults, larvae and pupae were counted. The results indicate that isolate DW B941 produced a 50% of larvae and workers mortality after 12 and 17 days, respectively. After 23 days 100% of workers were dead. Those values were different (p= 0.012) to the control. On wild nest the insect traffic decreased from 66 to 99% when they were fed with *B. bassiana* baits. After dissecting the nests, the treated ones showed a reduction of 51 and 71% of eggs and larvae, respectively. Furthermore, 76% of the remaining larvae were colonized by *B. bassiana,* instead any eggs was affected by the fungus.

Contributed Paper - Thursday, 16:45 167

Persistence and efficacy of entomopathogens in potting media.

Anne Nielsen - University of California at Davis, Davis, CA, USA; ***Kenneth Spence*** - University of California at Davis, Davis, CA, USA; ***Denny Bruck*** - USDA-ARS, Corvallis, OR, USA; ***Edwin Lewis*** - University of California at Davis, Davis, CA, USA
Address for correspondence: alnielsen@ucdavis.edu

Greenhouse nursery production represents a large and growing industry. In recent years there has been a shift both toward the use of soilless media (i.e. potting media) and integrated pest management. Application of entomopathogens such as nematodes, fungi, and bacteria against soil-dwelling insect pests is a sustainable alternative to insecticide centered control programs, but soilless media are designed to provide a plant pathogen free growing environment. The potential impact of soilless media on the efficacy of entomopathogens is often overlooked. We measured the effect of soilless media and watering regime on entomopathogen persistence under greenhouse conditions. The entomopathogens *Steinernema riobrave*, *Heterorhabditis bacteriophora*, and *Metarhizium anisopliae* were evaluated in three potting media; peat:sand mix, redwood bark mix and redwood sawdust mix. Aqueous soil drench and cadaver application methods were compared for *S. riobrave* and *H. bacteriophora*. *M. anisopliae* was applied as a soil drench. Survivorship decreased over the 4 week sampling period, independent of potting media or pathogen, although *M. anisopliae* had the longest persistence of all pathogens. The relationship between watering regime and persistence in media types was unclear. The persistence of entomopathogenic nematodes varied significantly among media types, but the best media type for each species and application method varied. The sawdust mix had the shortest persistence of all media tested and was not evaluated for efficacy. Efficacy of each pathogen was evaluated against *Otiorhynchus sulcatus* larvae in the best media from the persistence study (for each pathogen) 3 DAT and 10 DAT.

Contributed Paper - Thursday, 17:00 168

Field efficacy of a baculovirus isolate that doesn't cause the liquefaction of *Spodoptera frugiperda* dead larvae.

Fernando Valicente - Embrapa, Sete Lagoas, MG, Brazil; ***Corina Macedo*** - Student, Sete Lagoas, MG, Brazil; ***José Wolff*** - Universidade Presbiteriana Mackenzie, São Paulo, SP, Brazil; ***Edmar Tuelher*** - Student-Embrapa, Sete Lagoas, MG, Brazil; Carlos Paiva - Student-Embrapa, Sete Lagoas, MG, Brazil; ***Alan Costa*** - Student- Embrapa
Address for correspondence: valicent@cnpms.embrapa.br

The Spodoptera frugiperda nucleopolyhedrovirus (SfMNPV) has shown potential to be used in Brazil as a biopesticide. However, the liquefaction of the integument (isolate 19) makes large-scale production laborious and expensive, because all larvae must be frozen before being harvested for polyhedra extraction. One dead larva was found with the integument not disrupted (isolate 6) and was multiplied during 5 generations in laboratory. Detection and sequencing of chitinase and cathepsin genes were performed as well as LC_{50}, LT_{50} and field experiment using a wettable

powder formulation. The new Brazilian isolate 6 of S. frugiperda that doesn't disrupt the integument was confirmed to harbour cathepsin and chitinase genes. Restriction fragment analysis with BamHI and HindIII did not show differences between isolate 19 and 6. PCR amplification of the regions encompassing the chitinase and the cathepsin genes produced an amplicon whose size was the same for the two isolates. Alignment of the sequence (isolate 6) obtained with the sequence of isolate 19 revealed a deletion of one base located within the chitinase gene. The framesift caused by this deletion resulted in appearance of a stop codon 15 base pairs downstream the mutation. LC 50 was similar to both isolates (2.6x10 5 and 3.6x105 PIB/mL to isolate 6 and 19, respectively) but LT_{50} was around three days longer to isolate 6 than 19 on those concentrations equivalent to LC_{50} and LC_{95}. Field experiments using $1x10^7$ PIBs/mL showed mortality up to 90% when larvae were collected 24 and 48 hours after the bioinsecticide was sprayed. Mortality caused by baculovirus plus parasitoids was above 90% when a concentration of $1x10^6$ and $1x10^7$ PIB/mL of both isolates were used Using isolate 6, the larval equivalent/ha could be lowered to around 80 to 120 larvae/ha, which is equivalent to 10.75 and 13.86 g of dead larvae/ha, respectively.

Contributed Paper - Thursday, 17:15 169

Successful introduction of Green Muscle® into Madagascar for the control of the migratory locust *Locusta migratoria* Capito.

Jocelyn J.H. Rajaonarison - FOFIFA, Antananarivo, Madagascar; ***Nguya Kalemba Maniania*** - International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya; ***Rahalivavololona Njaka*** - FOFIFA, Antananarivo, Madagascar; ***Saholy Ramiliarijaona*** - FOFIFA, Antananarivo, Madagascar
Address for correspondence: nmaniania@icipe.org

The Malagasy migratory locust, *Locusta migratoria capito*, is a major threat to food production in Madagascar. Crop losses can be as high as 100% in some areas during severe outbreaks. The current control method based on the use of synthetic insecticides in plague or outbreak situations is costly, pollutes the environment and threatens Malagasy highly valued rich biodiversity. The use of *Metarhizium anisopliae* var. *acridum* is one of the alternatives being explored. Since Green Muscle® developed for the control of *Schistocerca gregaria* is now commercially available; steps were taken for its introduction into Madagascar. The steps included efficacy against *L. migratoria* in the laboratory and its effect on non-target organisms. Green Muscle® was virulent against *L. migratoria* nymphs and did not show negative effects on *Apis mellifica, Bombyx mori, Papillio demodecus* and *Antherina suraka*, and was therefore allowed into Madagascar for further studies. In field trials conducted in 2008 and 2009 in Tuléar Province, 5 and 16 ha, respectively, were treated with an ULV formulation of Green Muscled® at a rate of 100g conidia in 2000 mL of oil-kerosene/ha. Field populations of the locust were reduced to 70 and 80% 6 days after treatment during 2008 and 2009, respectively, while 100% reduction was achieved after 9 days post-treatment. Insects collected from treated plots at different interval times and placed in outdoor cages, all succumbed to fungal infection. Mortality of 100% was also observed after 25 days among nymphs that were exposed to treated grass collected 6 days post-treatment. These results confirm the potential of Green Muscle® as biopesticide for control of both *S. gregaria* and *L. migratoria*.

Contributed Paper - Thursday, 17:30 170

Beauveria bassiana UV resistance in the laboratory and its virulence against the coffee berry borer in the field.

Sandra Valdes - Cenicafé. National Centre of Coffee Research., Chinchina., Caldas, Colombia; ***Carmenza E Gongora*** - Cenicafe. National Centre of Cofee Research., Chinchina., Caldas., Colombia.
Address for correspondence: carmenza.gongora@cafedecolombia.com

The entomopathogenic fungus *Beauveria bassiana* displays a great potential as biological controller of insects. Nevertheless, the effectiveness of this microorganism depends to a large extent on its persistence under field conditions, which is affected by the solar radiation, particularly by UV-A and UV-B wavelengths. In studies under laboratory and field conditions it has been found that the highest coffee berry borer (CBB) mortality in laboratory conditions (100 %) is caused by a mixture of strains (Bb 9001, Bb9024 and Bb9119), that individually are low virulent strains, compared with the strain Bb 9205 considered a high virulence strain (88% mortality). However, under field conditions a good performance on CBB was exhibited by both, the mixture (67%) as well as the Bb 9205 (60%). In order to know the possible causes for this behavior, an evaluation of UV light resistance of the strains was done. Three ARSEF strains were tested (Bb718, Bb1053 and Bb2997), in addition to the individual low virulence strains (Bb 9001, Bb9024 and Bb9119), the mixture of these three, and the high virulence strain Bb 9205. All the strains were exposed to UV-A radiation, followed by UV-B and visible light by 15 min. periods. A higher percentage of resistance to UV light was found in the strain Bb 9205 compared to the mixture of low virulence strains, which is perhaps favoring the Bb 9205 strain under field conditions. On the other hand, the mixture showed a high potential because a lower concentration of spores is required to cause the highest mortality. A good formulation of this mixture will improve its field UV resistance and also virulence. This work has been co-financed by the Ministry of Agriculture and Rural Development of Colombia.

Contributed Paper - Thursday, 17:45 171

Biological control of Asian corn borer using *Wolbachia* infected line of *Trichogramma dendrolimi* and its evaluation.

Bin Cong - Laboratory for Biological Control, Shenyang Agricultural University, Shenyang, Liaoning, China ; ***Xihua Wang*** - Laboratory for Biological Control, Shenyang Agricultural University, Shenyang, Liaoning, China ; ***Haiyan Zhang*** - Laboratory for Biological Control, Shenyang Agricultural University, Shenyang, Liaoning, China ; ***Haitao Qian*** - Laboratory for Biological Control, Shenyang Agricultural University, Shenyang, Liaoning, China ; ***Hui Dong*** - Laboratory for Biological Control, Shenyang Agricultural University, Shenyang, Liaoning, China
Address for correspondence: bin1956@163.com

One new line was created by mean of horizontal transmission of a symbiont, *Wolbachia* from the donate wasp of *Trichogramma embryophagum* to the receptor wasp of *T. dendrolimi*. The reproduction pattern of *T. dendrolimi* was converted from arrhenotoky to thelytoky. A series trial was carried out to test the possibility to apply the new line in commercial mass-rearing and biological control. The comparison of the fecundity, developmental duration, Genetic stability, survival rate and dispersion, bionomics and ecological effects between the thelytokous line and the non-thelytokous line was conducted. The result showed that the tested trait was most similar; no significant difference was observed. A field release trial compared for two lines in 2007 and 2008, respectively, no significant difference was observed, again. These results imply that the thelytokous *T. dendrolimi* is a good prospect for the biological control of the asian corn borer (*Ostrinia furnacalis*) in northern China, where the *T.dendrolimi* had been released consistently on a large scale for over thirty years. The cost of mass rearing *Trichogramma* will be reduce by 20-30 percent if no male thelytoky line in used.

CONTRIBUTED PAPERS Thursday, 16:00 - 17:45
Painted Horse I-II

Bacteria III

Moderator: Juan Luis Jurat-Fuentes

Contributed Paper - Thursday, 16:00 172

Pathogenicity island in the Mexican *Serratia entomophila* mor4.1 active against *Phyllophaga blanchardi* larvae (Coleoptera).

Maria Eugenia Nuñez-Valdez - Facultad De Ciencias Agropecuarias, Universidad Autonoma del Estado de Morelos, Cuernavaca, Morelos, Mexico; ***Jean Chen*** - Depts. of Cell Biology and Neuroscience3, University of California, Riverside, Riverside, California, USA; ***Sarjeet Gill*** - Depts. of Cell Biology and Neuroscience, University of California, Riverside, Riverside, California, USA; ***Francisco Villalobos*** - Facultad De Ciencias Agropecuarias, Universidad Autonoma del Estado de Morelos, Cuernavaca, Morelos, Mexico; ***Zitlhally Rodriguez-Segura*** - Centro de Investigación en Biotecnología2, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico
Address for correspondence: eugenia@uaem.mx

The bacteria *S. entomophila* strain Mor4.1 (SeMor4.1; Enterobacteriaceae) was isolated in México from the haemocoel of a *Phyllophaga blanchardi* larvae. The bacteria is pathogenic to several species of the *Phyllophaga* genus (Coleoptera:Scarabaeidae; Nuñez- Valdez et al., 2008). These larvae feed on plant roots causing severe damage to some important crops world wide. The oral infection by *SeMor4.1* causes anti-feeding effect (AFE) and mortality. Insecticidal activity against *Anomala* sp and the lepidopteran *Manduca sexta* has been observed in bio-assays either by injecting the bacteria or by injecting cell free culture broths (Nuñez-Valdez et al., 2008 Appl. Environ. Microbiol. 74:802-10). We think that strain *Mor4.1* produce virulence factors with a wide spectrum of action, with toxic activities at the level of the insect gut and also at the level of the hemocoel. To identify the virulence factors, a genomic approach was followed by constructing an *S. entomophila Mor4.1* fosmid library in *E. coli*. The library clones expressing insecticidal activity by injection bio-assays were selected. We present the analysis of the DNA sequence of the 40 Kb of the clone named G8. We found that 19 genes were associated with virulence factors and some of them were located on a potential "Pathogenicity Island". Most ORFs (27) showed homology to *Serratia proteamaculans 568* proteins following almost the same gene order compared with *S. proteamaculans* genome, but with insertions of other genes in five positions. Two main virulence regions were identified in G8 i) a putative Lipopolysacharide (LPS) biosynthesis core (ORFs 29-40) and ii) a ppGpp gene operon (ORFs 9-13). There are evidences in other pathogenic bacteria, suggesting that the LPS (Kurz et al., 2003, EMBO J., 22:1451-1460) and the signaling molecule Guanosine Tetraphosphate ppGpp (Nakanishi et al., Molecular Microbiology 61(1), 194–205) might work as potential virulence factors in *SeMor4.1* to scarab larvae.

Contributed Paper - Thursday, 16:15 173

From insect to man: A functional genomic comparison of clinical and insect pathogenic strains of *Photorhabdus.*

Maria Sanchez-Contreras - University of Bath, Banes, UK; ***Andrea Dowling*** - University of Exeter, Penryn, Cornwall, UK; ***Richard ffrench-Constant*** - University of Exeter, Penryn, Cornwall, UK; ***Nick Waterfield*** - University of Bath, Banes, UK
Address for correspondence: bssnw@bath.ac.uk

Bioinformatics is becoming increasingly successful at the annotation of genomes and yet it still cannot detect novel virulence genes or prove functionality. With this in mind we developed a powerful technique named Rapid Virulence Annotation (RVA). RVA employs the parallel screening of large insert DNA libraries of a bacterial pathogen of choice, against multiple invertebrate hosts. The use of these "gain of toxicity" screens in otherwise harmless *E. coli* circumvents certain problems inherent in traditional gene knock-out screens. These include avoiding the issue of virulence gene redundancy, revealing less potent factors that may otherwise be masked by dominant toxins and the detection of the final virulence effectors rather than pleiotrophic regulators. We screen libraries against the nematode Caenorhabditis elegans, serving as an oral route model, the single-cell protozoa *Acanthamoeba polyphaga* used as a phagocytosis model, and two caterpillar models, the Tobacco hornworm *Manduca sexta* and the Waxmoth *Galleria mellonella,* both of which represent the more complex innate immune systems. Finally, we use the mouse BALB/c macrophage cell line J774-2 to represent the phagocytic component of the vertebrate immune system. The genus *Photorhabdus* can be split into 3 species based on multi-locus sequence typing. All three of these species exist in a symbiotic nematode-bacterial complex with an insect pathogenic nematode worm of the genus *Heterorhabditis.* Two of these species, *P. luminescens* and *P. temperata* are exclusive insect pathogens while the third, *P. asymbiotica,* is both an insect and human pathogen and may be considered an "emerging" human pathogen. We present a comparison of an RVA analysis of the two sequenced strains, P. luminescens TT01, an insect only pathogen, and the clinical isolate P. asymbiotica ATCC43949. We will discuss the communality of virulence factors and the implications for insect pathogens as a potential source of virulence factors and emerging infections in mammals.

Contributed Paper - Thursday, 16:30 174

The fate of toxin complexes in cultured cells.

Michelle Hares - University of Exeter, Penryn, Cornwall, U.K.; ***Stewart Hinchliffe*** - University of Exeter, Penryn, Cornwall, U.K.; ***Richard ffrench-Constant*** - University of Exeter, Penryn, Cornwall, U.K.
Address for correspondence: m.c.hares@exeter.ac.uk

The Toxin complex (Tc) genes were first identified in the insect pathogen *Photorhabdus luminescens* strain W14. These high molecular weight proteins (~1MDa) have been shown to be orally and injectably toxic to several orders of insects. They are encoded by four loci; tca, tcb, tcc and tcd, the genes within these loci labelled according to their order (tcdA, tcbB, tccC). Significant homology is observed between the loci and previous work has shown that three components are required for full toxicity, the tcdA-like [A], the tcbB-like [B] and tccC-like [C] genes. Interestingly these Tc's are seen in a variety of gram-negative pathogenic bacteria including Yersinia, suggesting an evolving function or targets for these proteins directed towards insect and /or mammalian hosts. Previous work has show that when the Tc's are applied to cultured cells membrane ruffling, vacuolation and multinucleation is induced. Transfection of A, B and C into these cells determined that A was responsible for multinucleation, B induced vacuolation, whereas C had no effect. Using GFP-tagged A, B and C proteins in combination with immunofluorescent markers for membrane trafficking compartments we present work to demonstrate the origin of these vacuoles and the fate of the Tc's.

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Bacillus thuringiensis biopesticide produced with different amounts of carbon and nitrogen.

Fernando Valicente - Embrapa Maize and Sorghum, Sete Lagoas, Minas

Gerais, Brazil; ***Andre Horta*** - Agronomy Student, Jaboticabal, São Paulo, Brazil; ***Lucas Mendes*** - Agronomy Student, Patos de Minas, MG, Brazil; ***Amanda Lanza*** - Student, Sete Lagoas, MG, Brazil; ***Sandra Miranda*** - Student, Sete Lagoas, MG, Brazil
Address for correspondence: valicent@cnpms.embrapa.br

The amount of carbon and nitrogen used to produce *Bacillus thuringiensis* biopesticide may influence the quality of the final product. This research used different levels of carbon and nitrogen in 3 bioassays with 5 treatments each, and LB medium was the check treatment. The first bioassay used 5g/L of maize glucose for all treatments with yeast ranging from 5g/L to 60g/L. The second bioassay used 30g/L of yeast for all treatments with maize glucose ranging from 5g/L to 60g/L. The third bioassay used increasing amounts of nutrient ranging from 1g/L of maize glucose and 3g/L of yeast up to 20g/L of maize glucose and 60g/L of yeast. All media were enriched with salts (FeSO4, ZnSO4, MnSO4, MgSO4). The seed culture was produced using LB medium plus salts, at a stirrer speed of 200rpm, for 18 hours at 30ºC. All media were sterilized and inoculated with Bt strain 344 (*B. thuringiensis tolworthi*) and maintained at 30ºC for 72 hours at a stirrer speed of 250rpm. The pH was measured at regular intervals, heat resistant spores were expressed as c.f.u/mL, cell mass produced in g/L-lyophilized, and spore counting per mL of medium. Results showed that pH followed the same pattern for all media tested, decreasing in the first 12-14 hours and increasing up to 8.7 (no pH control was made). The number of spores reached 4.9 x 109 spores/mL, and the lowest amount of 1.09 x 109 spores/mL. In the second bioassay the maximum number of spores was reached within 48h. Cumulative cell mass produced more than 30.0g/L in many treatments were the amount of nitrogen was higher. Mortality of 2-day-old *Spodoptera frugiperda* larvae was a 100% when treated with spores withdrawn at 24 hours from bioassay 3, and a 100% after 48 hours with spores withdrawn from bioassay 2 and 3.

Contributed Paper - Thursday, 17:00 176

Expression of aminopeptidases in *Ostrinia nubilalis* (Hübner).

Maria Cristina Crava - Dep. Genética. Universitat de Valencia, Burjassot, Valencia, Spain; ***Yolanda Bel*** - Dep. Genética. Universitat de Valencia, Burjassot, Valencia, Spain; ***Barbara Manachini*** - Dep. Animal Biology. U. Palermo, Palermo, Italy; ***Juan Ferré*** - Dep. Genética. Universitat de Valencia, Burjassot, Valencia, Spain; ***Baltasar Escriche*** - Dep. Genética. Universitat de Valencia, Burjassot, Valencia, Spain
Address for correspondence: baltasar.escriche@uv.es

The aminopeptidases N (APNs) are a large family of enzymes with probable role in food digestion that have been detected in the midgut of several lepidopteran species. Aside of their insect physiological role, they have become relevant because of their function as membrane binding proteins involved in the mode of action of *Bacillus thuringiensis* (Bt) crystal protein biopesticidal toxins. In the present study, the expressions of 5 *apn* genes and *one puromicyne-sensitive aminopeptidase* (*psa*) gene have been characterized in *Ostrinia nubilalis* (Hübner), a key pest of Bt-corn The analysis by RT-PCR showed that all aminopeptidases were expressed along the whole larval development. The relative tissue expression analyses in 5th instar larvae by qRT-PCR showed that all aminopeptidase genes were transcribed in the midgut. Moreover, 2 *apns* (*Onapn4* and *Onapn8*) were also expressed in Malpighian tubules, and the *Onpsa* transcripts were found at similar levels in those tissues as well as in the fat body and carcass. The *Onapn8* was expressed in the Malpighian tubules and in the midgut tissue without statistically significant differences, whereas the *Onapn4* had a very low level of expression in the Malpighian tubules. The *in silico* structural putative aminoacidic sequence differences between APNs and PSA seems to be correlated with their expression patterns. The structural similarity and expression of the analyzed APNs suggest that more than a single class may be involved in the Bt toxin binding in the migut.

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Characterization, distribution and cloning *cry1* genes efficient against fall armyworm, *Spodoptera frugiperda*, in Brazil.

Fernando Valicente - Embrapa, Sete Lagoas, MG, Brazil; ***Edgard Picoli*** - Post Doc / Embrapa, Sete lagoas, MG, Brazil; ***Maria Vasconcelos*** - Embrapa, Sete Lagoas, MG, Brazil; ***Newton Carneiro*** - Embrapa, Sete Lagoas, MG, Brazil; ***Andrea Carneiro*** - Embrapa - Sete Lagoas, MG, Brazil; ***Cláudia T. Guimarães*** - Embrapa Sete Lagoas, MG, Brazil; ***Ubiraci G. P. Lana*** - Embrapa, Sete Lagoas, MG, Brazil
Address for correspondence: valicent@cnpms.embrapa.br

Brazil is located in South America and contains a rich and different biodiversity. A total of 4,459 Bt strains were isolated and evaluated regarding to *Spodoptera frugiperda* larval mortality, and 165 showed larval mortality above 75%. Molecular characterization was based on PCR electrophoresis profile using specific cry1 primers. Among these strains, 33 (20%) did not amplify the expected fragments; 103 (62,42%) amplified fragments corresponding to the presence of only one gene, while 25 (15,15%), 3 (1,8%) and 1 (0,6%) showed a profile of two, three and four different cry1 genes, respectively. SDS-page protein analyses were positive for the presence of cry1 genes. The most frequent (57.5%) was cry1D gene, whereas cry1Aa/cry1Ad and cry1C genes were the less frequent (1.2%). However, more than 60% of the evaluated strains presented cry1B and cry1E genes. Analysis of strains carrying cry1C, cry1B, cry1E, cry1F, cry1A, cry1G and cry1D genes showed that they were toxic to *S. frugiperda,* ranging from the most to the least toxic. The available sequences at http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/ were used for designing primers to clone cry1C and cry1F genes. The amplified fragments with the expected size, approximately 2,046 bp, were purified, cloned and transformed into competent cells. The sequencing of 5' and 3' ends allowed the confirmation of the identity of the genes. Some strains that presented unspecific fragments, were also cloned, amplified, sequenced and showed sequences corresponding to cry1-type genes. Colonies holding clones of cry1F, cry1Ca and cry1Cb genes, were obtained only for two of the evaluated strains.

Contributed Paper - Thursday, 17:30 178

Plasmid capture system and its applications.

Jong Yul Roh - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Yong Wang*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Qin Liu*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Xueying Tao*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Jae Young Choi*** - Research Institute for Agriculture and Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Hee Jin Shim*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea; ***Hong Guang Xu*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, ***Soo Dong Woo*** - Department of

Agricultural Biology Chungbuk National University, Korea, ***Byung Rae Jin*** - College of Natural Resources and Life Science, Dong-A University, Korea, ***Yeon Ho Je*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea
Address for correspondence: rohling@snu.ac.kr

Plasmid capture system (PCS) was developed for easy cloning and manipulation of circular double-stranded DNA from various sources. Recently, we improved PCS system (named PCS-LZ) to clone relatively large-sized DNA molecules (30~150 kb). PCS-LZ donor consists of a Mini-F replicon and a kanamycin resistance marker between Tn7L and Tn7R regions. Both replicon and marker gene of PCS-LZ donor are transferred into target plasmid DNAs by *in vitro* transposition and the transposed DNAs can replicate in *E. coli* cells by transformation. White/blue screening by LacZ expression is also available to avoid backgrounds. Up to now, we acquired various circular DNA clones from four sources such as plasmids of *B. thuringiensis*, bacteriophage genome isolated from *B. thuringiensis*, genome segments of *Cotesia glomerata* bracovirus, and polymorphic genomes of *Autographa californica* nucleopolyhedrovirus. Among them, interestingly, the genome clones of bacteriphage (Ph1-3) were screened from the PCS transposition with plasmids of *B. thuringiensis* 1-3 strain. The genome of Ph1-3 was fully sequenced (46517 bp) and open reading frames were analyzed. In accordance with this genome finding, the phage particles and its DNA were confirmed from the supernatant of *B. thuringiensis* 1-3. Ph1-3 showed infectivity to *B. thuringiensis* type strains such as subsp. *galleriae*, *entomocidus*, and *morrisoni*. Based on these results, we screened the existence of phage in *B. thuringiensis* type strains by PCR with terminase small subunit-specific primers. Ten of 67 type strains showed PCR products and their sequence similarity was more than 70%. Conclusively, we expect this PCS-LZ system would be a powerful tool for genomic analysis and mutagenesis study at the area of invertebrate pathology and further its application will be enlarged to the vertebrate pathology area.

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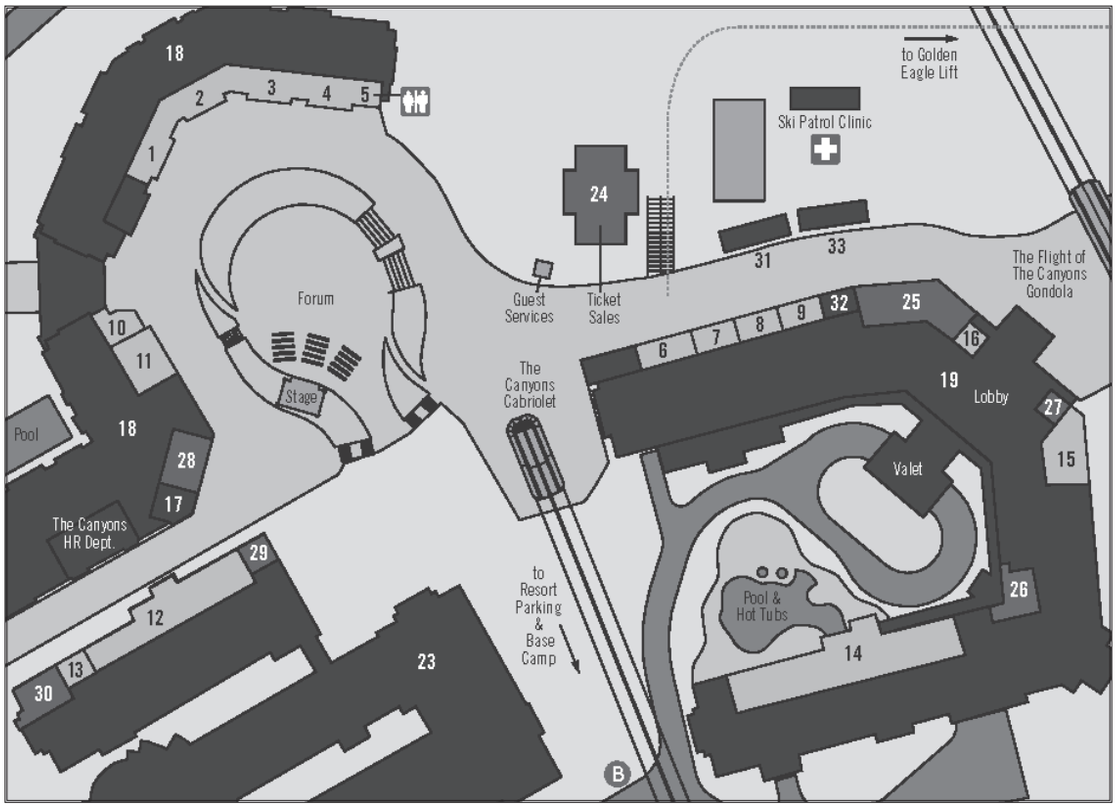
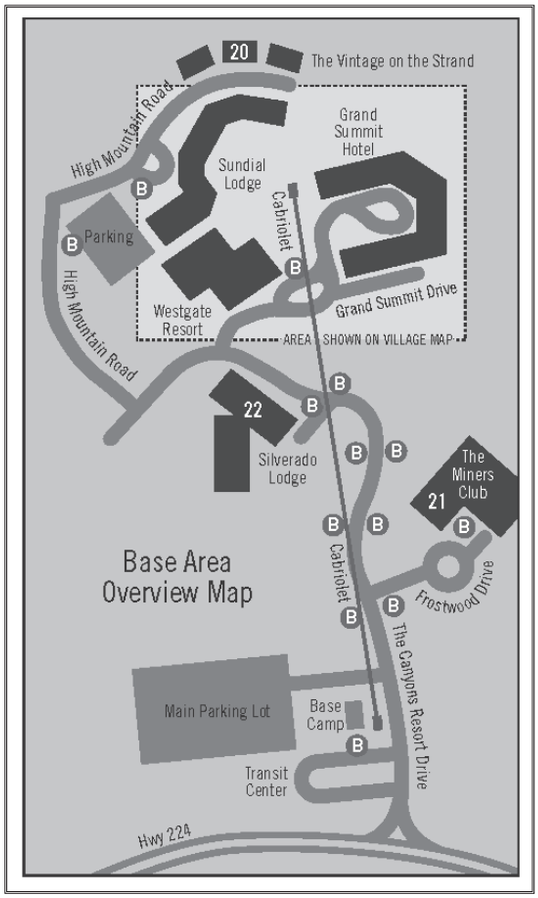




SHOPS/SERVICES

1. One Sweet Ride
2. Canyon Mountain Sports
3. Canyon Mountain Rentals
4. Solid Edge Tuning & Repair
5. Daylodge/Lockers/Restrooms
6. The Canyons Resort Realty
7. Surefoot
8. Canyon Mountain Sports at The Grand Summit
9. Ski Check/SharpShooter Images
10. Kindersport
11. The Glory Hole
12. Papillion, The Spa
13. Aloha Rental Shop
14. Grand Summit Health Club & Spa
15. Little Adventures Daycare
16. Grand Summit General Store (lobby level)

B Bus Stop

LODGING

17. The Canyons Lodging Office
18. Sundial Lodge "B" and "C"
19. The Canyons Grand Summit Hotel
20. The Vintage on the Strand
21. The Miners Club
22. Silverado Lodge
23. Westgate Resort

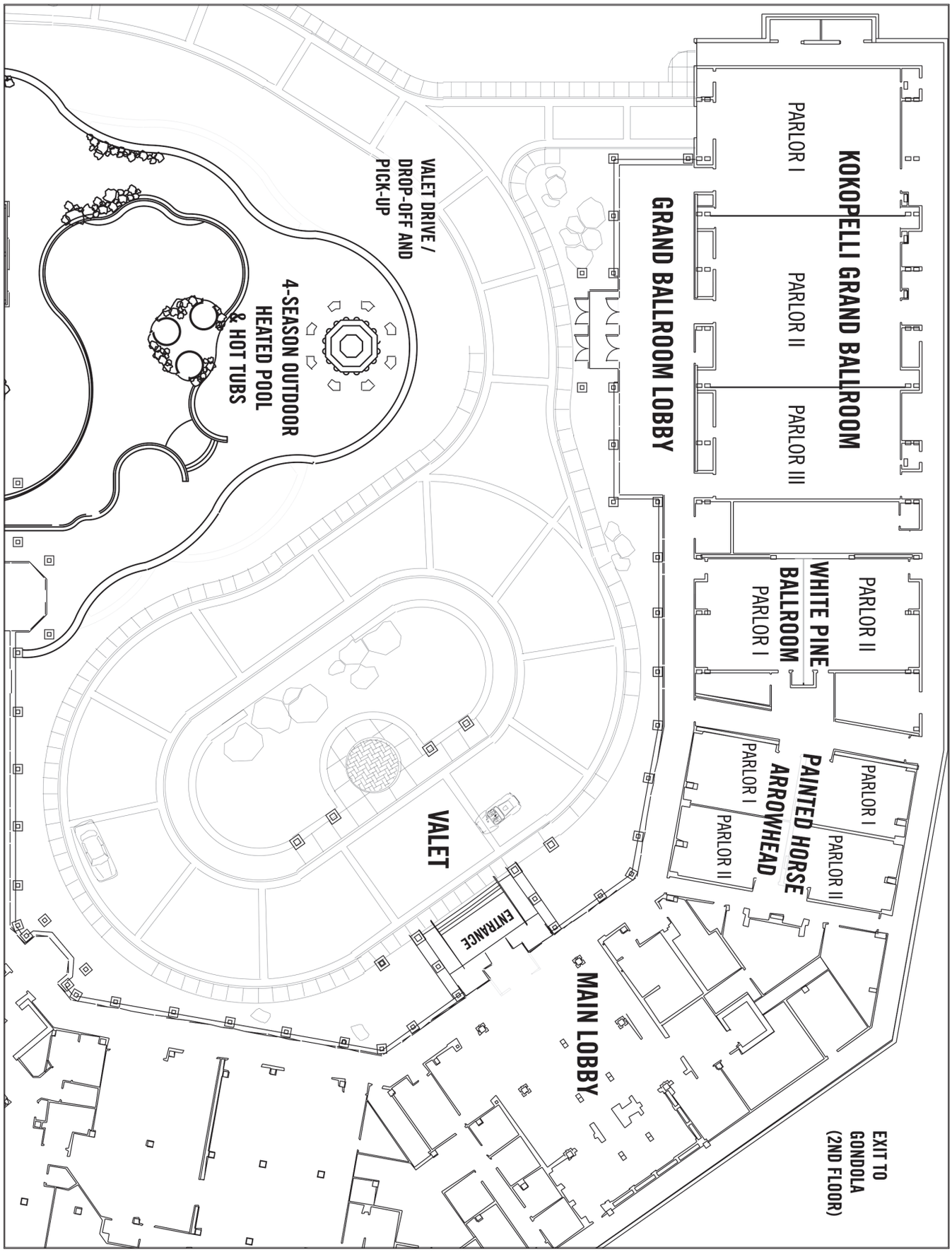
RESTAURANTS/BARS

24. Smokie's Bar & Grill
25. Doc's at the Gondola
26. The Cabin Restaurant and Lounge (lobby level)
27. First Tracks Café (lobby level)
28. Powder Daze Café & Creperie
29. Westgate Marketplace & Deli
30. Westgate Grill

SKI SCHOOL

31. The Canyons Ski & Snowboard School Sales Cabin
32. Children's Ski & Snowboard School Pick-up/Drop-off
33. Adult Ski & Snowboard School Rentals

PARK CITY AREA MAP


KOKOPELLI GRAND BALLROOM
PARLOR I
PARLOR II
PARLOR III
GRAND BALLROOM LOBBY
WHITE PINE BALLROOM
PARLOR I
PARLOR II
PAINTED HORSE
ARROWHEAD
PARLOR I
PARLOR II
PARLOR I
PARLOR II
VALET DRIVE / DROP-OFF AND PICK-UP
4-SEASON OUTDOOR HEATED POOL & HOT TUBS
VALET
ENTRANCE
MAIN LOBBY
EXIT TO GONDOLA (2ND FLOOR)

All meetings and all meals will be held at the Grand Summit Hotel. Your conference badge is your entrance and meal ticket, please keep it with you.

Agenda continued from front inside cover

10:30 – 12:30	**Contributed Papers:**	
	*Nematodes II	*Painted Horse*
	*Microbial Control I	*White Pine*
	*Virus II	*Ballroom III*
12:30 – 14:00	Lunch Buffet	*Outdoor Pavillion*
12:30 – 14:00	**Student Workshop & Lunch**	
	*How to Get a Postdoc Position and Get Into the Scientific Network	*Painted Horse*
14:00 – 16:00	**Symposium:**	
	*Insect Defense Responses to Fungal Pathogens	*Ballroom III*
14:00 – 16:00	**Contributed Papers:**	
	*Microbial Control II	*White Pine*
	*Virus III	*Ballroom II*
16:00 – 16:30	Break	*Kokopelli Lobby*
16:30 – 17:30	**POSTER 2 -** Beneficial Invertebrates, Fungi, Microsporidia, Virus	*Ballroom I*
17:30 – 19:00	Dinner Buffet	*Outdoor Pavillion*
19:00 – 20:00	**Division Business Meetings**	
	*Microbial Control Division	*Ballroom II*
	*Pathogens of Beneficial Invertebrates Division	*White Pine*
20:00 – 21:00	**Beneficial Invertebrates Division Workshop**	
	*Bee Health—Diseases and Cures	*White Pine*

Thursday – 20 August

6:30 – 8:00	Breakfast Buffet	*Outdoor Pavillion*
7:00 – 12:00	Registration Open	*Kokopelli Lobby*
8:00 – 10:00	**Symposia**:	
	*Biopesticides in Organic Farming: Available and Potential Technologies	*White Pine*
	*Microsporidia of Beneficial Arthropods	*Painted Horse*
8:00 – 10:00	**Contributed Papers:**	
	*Virus IV	*Ballroom III*
10:00 – 10:15	Break	*Kokopelli Lobby*
10:15 – 12:30	**SIP Annual Business Meeting**	*Ballroom*
12:00 – 13:30	Lunch Buffet	*Outdoor Pavillion*
12:00 – 13:30	Student Business Meeting & Lunch	*Doc's*
12:00 – 13:30	Student Awards Committee Meeting & Lunch	*The Cabin*
13:30 – 15:30	**Symposia:**	
	*The Viral Face of PDV's: Origin and Structure of the Chromosomally Integrated PDV Genomes	*Ballroom III*
	*Ecological Interactions in Entomopathogenic Nematodes	*Painted Horse*
13:30 – 15:30	**Contributed Papers:**	
	*Microbial Control III	*White Pine*
15:30 – 16:00	Break	*Kokopelli Lobby*
16:00 – 18:00	**Symposium**	
	*Multitrophic Interactions: Implications for Invertebrate Pathogens	*Ballroom II*
16:00 – 18:00	**Contributed Papers:**	
	*Microbial Control IV	*White Pine*
	*Bacteria III	*Painted Horse*
19:00 – 20:00	COCKTAIL HOUR	*Kokopelli Lobby*
20:00	BANQUET & AWARDS CEREMONY	*Ballroom*

IMPORTANT NOTE ABOUT POSTERS

Posters should be displayed by 8:00 Monday or Wednesday in Kokopelli I. Posters must be removed no later than 20:00 on Tuesday and 15:30 on Thursday. Presenters should stand by their posters during the appropriate poster session.

MEALS

Meals are paid for in advance, and are included in the registration fee. You will need to show your conference badge to restaurant staff.

The support of the following organizations for the
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Bayer CropScience

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