46th Annual Meeting of the Society for Invertebrate Pathology Conference on Invertebrate Pathology and Microbial Control

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NEMASYM RCN
Nematode-Bacterium Symbioses Research Coordination Network
5th NEMASYM Meeting

11 – 15 August 2013
Pittsburgh, Pennsylvania, USA
Program and Abstracts

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PROGRAM

2013

IMPORTANT NOTES:

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

STU indicates papers being judged for graduate student presentation awards

SUNDAY - 11 August

08:00–17:00	SIP Council Meeting	**Stoops Ferry**
11:00–21:00	Registration	**Lobby**
18:00–21:00	Mixer	**Reflections/Waterfront**
14:00–21:00	Put up posters	**Grand Station III–V**

MONDAY - 12 August

07:00–17:00 Registration **Lobby**

07:00–08:30 **BREAKFAST** Grand Station III-V

Monday, 08:30-10:00.
Grand Station I-II

Opening Ceremonies and SIP Founders' Memorial Lecture

Opening Ceremonies

Nina Jenkins, Chair, Organizing Committee
Jørgen Eilenberg, President, SIP

Founders' Memorial Lecture

Baculoviruses and their interactions with insects. From basic biology to biotechnology in the –omics era: the immense contributions of Bob Granados

Introduced by James Becnel
Honoree: **ROBERT GRANADOS**
Lecturer: **GARY BLISSARD**

10:00–10:25 **BREAK** Grand Station III-V

Plenary Symposium — Monday, 10:30–12:30. Grand Station I-II

Novel perspectives on the ecology and evolution of host-pathogen interactions

Organizer/Moderator: Matt Thomas

10:30 **1** **Complexity in the Function and Evolution of Insect Immunity** Brian P. Lazzaro Dept. Entomology, Cornell University, Itheca, NY, USA.

11:00 **2** **Variation in heterogeneity of transmission helps maintain diversity in an insect viral pathogen**
Arietta Fleming-Davies[1], Vanja Dukic[2], Brian Rehill [3] and Greg Dwyer[1] [1] Ecology & Evolution, University of Chicago, 900 E 57th St, Chicago IL 60637, USA. [2] Dept. of Applied Mathematics, 526 UCB, University of Colorado, Boulder, CO 80309, USA. [3] Chemistry Dept., U.S. Naval Academy, 572M Holloway Road, Annapolis, MD 21402, USA. (arietta@uchicago.edu)

11:30 **3** **The role of environmental variability in shaping insect immunity and resistance.** Courtney Murdock Penn State University, University Park, PA, USA.

12:00 **4** **Friendly competition: what happens to the "dilution effect" when hosts compete?**
Spencer Hall[1], Alex Strauss[1], Meghan Duffy[2], and Carla Cáceres[3] [1]Department of Biology, Indiana University, Bloomington IN 47405 USA, sprhall@indiana.edu and atstraus@indiana.edu [2]Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109 USA, duffymeg@umich.edu [3]Department of Animal Biology, University of Illinois, Urbana, IL 61801 USA, caceres@life.illinois.edu

12:30–14:00 **LUNCH** On your own

Symposium — Monday, 14:00-16:00. Grand Station I-II
(Viruses & Diseases of Beneficial Invertebrates)

Invertebrate innate immunity

Organizer/Moderator: Lorena Passarelli & Elke Genersch

14:00 **5** **Regulation of hemolymph protease cascades in the immune system of *Manduca sexta***
Michael R. Kanost
Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS 66506 USA (kanost@ksu.edu)

14:30 **6** **Defense Responses of *Biomphalaria* to Schistosomes**
Eric S. Loker Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131, USA esloker@unm.edu

15:00 **7** **Multiple microbes and immunity in honey bees**
Jay D. Evans, Ryan S. Schwarz
USDA-ARS Bee Research Lab BARC-East Bldg 306 Beltsville, MD USA 20705 (jay.evans@ars.usda.gov)

15:30 **8** **Antiviral defense in aphids**
Bryony C. Bonning[1], Diveena Vijayendran[1], Sijun Liu[1]
[1]Department of Entomology, Iowa State University, Ames, IA 50011, USA. (bbonning@iastate.edu)

Contributed Papers — Monday, 14:00-16:00. Ellwood

Nematodes 1

Moderator: Edwin Lewis & Vladimir Půža

14:00 **9** **Entomopathogenic nematodes and soil food webs: Natural assemblages and specific niche associations in natural *versus* agricultural areas**
Raquel Campos-Herrera [1,2], Fahiem E. El-Borai [1,3], and Larry W. Duncan[1] [1] Departamento de Contaminación Ambiental, Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Ciencias Agrarias (ICA), 28006, Madrid (Spain), [2] Citrus Research and Education Center, University of Florida, 33850, Lake Alfred (Florida, USA), [3] Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt. (raquel.campos@ica.csic.es; r.camposherrera@ufl.edu)

14:15 **10** **Storage temperature and duration affect *Steinernema scarabaei* dispersal and attraction, virulence, and infectivity to a white grub host**
Albrecht M. Koppenhöfer, Lemma Ebssa, Eugene M. Fuzy
Department of Entomology, Rutgers University, New Brunswick, NJ 08901, USA. (koppenhofer@aesop.rutgers.edu)

14:30 **11** **Parasitism of *Sirex noctilio* by non-sterilizing *Deladenus siricidicola* in northeastern North America**
Stefanie A Kroll[1], E. Erin Morris[1], Stefan J. Long[1], and Ann E. Hajek[1] [1]Department of Entomology, Cornell University, Ithaca, New York 14853-2601, USA. (eem62@cornell.edu)

14:45 **12 STU** **Community composition of entomopathogenic nematodes associated with Vaccinium spp. roots in cultivated and wild settings**
Monique J. Rivera (Rutgers University, mjrivera@rutgers.edu)
Albrecht Koppenhöfer (Rutgers University,

koppenhofer@aesop.rutgers.edu) Cesar Rodriguez-Saona (Rutgers University, crodriguez@aesop.rutgers.edu)

15:00 **13 Influence of the Natural Microbiome on Nematode Growth in the Wild**
Buck S. Samuel[1], Holli Rowedder[1], Christian Braendle[2], Marie-Anne Felix[3], Gary Ruvkun[1] [1]Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA; [2] Institute of Developmental Biology and Cancer, CNRS, University of Nice Sophia-Antipolis, Nice, France; [3] Institute of Biology of the Ecole Normale Supérieure (IBENS), Paris, France (bsamuel@molbio.mgh.harvard.edu)

15:15 **14 Group behavior in insect parasitic nematode dispersal**
David I. Shapiro-Ilan[1], Edwin E. Lewis[2] and Paul Schliekelman[3] [1]USDA-ARS, SEFTNRL, 21 Dunbar Road., Byron, GA 31008 USA. [2]UC Davis, Department of Nematology, Department of Entomology, University of California, Davis, CA 95616 USA. [3]Department of Statistics, University of Georgia, Athens, GA USA 30602. (David.Shapiro@ars.uda.gov)

15:30 **15 Entomopathogenic nematode attraction to the chemical cues produced by cadavers.**
Johnson, Quanytta[1], Peabody, Alphe[1], Grochowski, Laura[1], Stevens, Glen[1] [1]Ferrum College, School of Natural Sciences and Mathematics, Ferrum, VA 24088 USA (gstevens3@ferrum.edu)

15:45 **16 STU Potential Natural Enemies of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae)**
[1]Derya Ulug, [1]Selcuk Hazir, [2]Harry K. Kaya, [2]Edwin Lewis [1] Adnan Menderes University, Faculty of Arts and Science, Department of Biology 09010 Aydin, TURKEY. (deryaasici@gmail.com). [2] Department of Entomology and Nematology, University of California, One Shields Avenue, Davis, CA 95616.

Contributed Papers — Monday, 14:00-16:00. Haselton

Bacteria 1

Moderators: Baltasar Escriche & William Moar

14:00 **17 Comprehensive analysis of gene expression profiles of the beet armyworm *Spodoptera exigua* larvae challenged with *Bacillus thuringiensis* Vip3Aa toxin**
Yolanda Bel[1], Agata K. Jakubowska[1], Juliana Costa[2], Salvador Herrero[1] and Baltasar Escriche[1] [1]Departament de Genètica, Universitat de València, 46100 Burjassot (Valencia), Spain. [2]Universidade Federal de Viçosa (UFV), Empresa Rizoflora Biotecnologia S/A, Caixa Postal 275, CEP 36570-000, Viçosa - MG,Brasil. (Yolanda.Bel@uv.es)

14:15 **18 Retargeting of the Bt toxin Cyt2Aa against hemipteran insect pests.**
Nanasaheb P. Chougule[1], Huarong Li[1,2], Sijun Liu[1], Lucas B, Linz[1], Kenneth E. Narva[2] , Thomas Meade[2], Bryony C. Bonning[1] [1]Department of Entomology, Iowa State University, Ames, Iowa, USA. [2]Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268, USA. (chougule@iastate.edu)

14:30 **19 Differential binding of Cry1Ab and Cry1Fa proteins from *Bacillus thuringiensis* to five aminopeptidases N from *Ostrinia nubilalis* (Hübner)**
M. Cristina Crava, Yolanda Bel, Agata Jakubowska, Juan Ferré and Baltasar Escriche Departament de Genètica, Universitat de València, 46100 Burjassot (Valencia), Spain. (baltasar.escriche@uv.es)

14:45 **20 Genome characterizing of mosquitocidal *Bacillus thuringensis* isolate S2160-1**
Hao Zhong [23], Yanjun Wei[4], Youzhi Li[3], Yan Zhang[4], Jim X. Fang[123] [1]The HITAR Institute Canada Inc., Richmond, British Columbia, Canada. [2]Hainan Institute of Tropical Agricultural resources, Sanya, 572025, Hainan, China.[3]College of Life and Technology Science, Guangxi University, Nanning, 530004, China. [4]College of Bioinformatics Science and Technology, Harbin Medical University, Harbin 150081, China (jim.xj.fang@hitar.org)

15:00 **21 A novel microcapsule formulation: alkaline releasing for *Bacillus thuringiensis* insecticidal proteins**
Shuyuan Guo[1], Wenhui Yang[1], Kanglai He[2] [1]School of Life Science, Beijing Institute of Technology, Beijing 100081, China [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China. (guosy@bit.edu.cn)

15:15 **22 Importance of alkaline phosphatase in the mode of action of Cry1 toxins**
Liang Gong[1,2], Zhaojiang Guo[3], Siva Jakka[1], Joel Sheets[4], James Hasler[4], Youjun Zhang[3], and Juan Luis Jurat-Fuentes[1] [1]Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA. [2]College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China. [3]Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China. [4]Dow AgroSciences, Indianapolis, IN 46268, USA. (jurat@utk.edu)

15:30 **23 Cross-order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins**
K. van Frankenhuyzen, Great Lakes Forestry Centre, Canadian Forest Service, Natural Resources Canada, 1219 Queen Street East, Sault Ste. Marie, Ontario, Canada P6A 2E5 (kvanfran@nrcan.gc.ca).

15:45 **24 STU The insecticidal specificity of Cry1Ah protein**
Zishan Zhou, Huiyan Lin, Changlong Shu, Fuping Song, Jie Zhang* State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China (zishanzhou@126.com)

Contributed Papers — Monday, 14:00-15:45. Fountainview

Fungi 1

Moderators: Fernando Vega & Nicolai vitt Meyling

14:00 **25 STU Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect pathogenic fungi**
Scott W. Behie and Michael J. Bidochka Department of Biological Sciences, Brock University, St. Catharines, ON Canada (sb07fh@brocku.ca)

14:15 **26 Evolutionary forces acting on endophytic insect pathogenic fungi**
Michael J. Bidochka and Scott W. Behie Department of Biological Sciences, Brock University, St. Catharines, ON Canada (bidochka@brocku.ca)

14:30 **27 Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae)**
Komivi S. Akutse[1,2], Nguya K. Maniania[1], Komi K.M. Fiaboe[1], Johnnie Van den Berg[2] and Sunday Ekesi[1] [1]International Centre of Insect Physiology and Ecology (*icipe*), P. O. Box 30772-00100, Nairobi, Kenya; [2] Unit of Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South

Africa (nmaniania@icipe.org)

14:45 **28 Overlapping gene functions in the endophytic insect-pathogenic fungus *Metarhizium*.**
Israel Enrique Padilla-Guerrero[1], Zaizy Rocha-Pino[1,2], Keiko Shirai[2] and Michael J Bidochka[1].
[1]Brock University, St. Catharines, ON, Canada; [2]Universidad Autonoma Metropolitana, Mexico City, Mexico. (isen28@hotmail.com)

15:00 **29 Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals plant pathogen-like effectors**
Quincy B. Conway, Norma C. Salazar, Ana Paula Delgado, Jhanelle K. Dawes, Lauren G. Douma and Aurélien Tartar
Nova Southeastern University, Fort Lauderdale, FL, USA (aurelien@nova.edu)

15:15 **30 Characterization of a G-protein coupled receptor that links carbon sensing to conidiation, blastospore development, stress resistance, and virulence in *Beauveria bassiana***
Sheng-Hua Ying[1,2*], Ming-Guang Feng[1], and Nemat O. Keyhani[2*] [1]Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, China, 310058 [2]Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611(yingsh@zju.edu.cn)

15:30 **31 STU Cell wall integrity pathway regulates *Beauveria bassiana* responses to developmental and stressful cues via crosstalk with HOG pathway**
Ying Chen, Sheng-Hua Ying and Ming-Guang Feng \
Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, People's Republic of China Address for correspondence: mgfeng@zju.edu.cn

16:00–16:30 **BREAK** Grand Station III-V

Symposium (Viruses) Monday, 16:30-18:30. Grand Station I-II

Evolution of traits and host usage by the related polydnaviruses, baculoviruses, nudiviruses, and salivary gland hypertrophy viurses

Organizers/Moderators: Mike Strand, Elisabeth Herniou, & Michel Cusson

16:30 **32 TBD** Just Vlak

16:50 **33 Comparative genomics and evolution of baculoviruses, nudiviruses and bracoviruses.**
Annie Bézier, Julien Thézé, Faustine Louis, Jean-Michel Drezen, Elisabeth A. Herniou
Institut de Recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François Rabelais, Parc de Grandmont, 37200 Tours, France. (annie.bezier@univ-tours.fr)

17:10 **34 Mutualistic Polydnaviruses Share Essential Replication Gene Functions with Pathogenic Ancestors**
Gaelen R. Burke, Sarah A. Thomas, Jai H. Eum, Michael R. Strand Department of Entomology, The University of Georgia (grburke@uga.edu)

17:30 **35 Functional Studies on the Hytrosaviridae: a large dsDNA Non-occluded Virus Infecting Adult Diptera**
D. G. Boucias[1], H. M. Karithi[2,3], and A. M M. Abd-Alla[2]
[1]Entomology and Nematology Department, Building 970, Natural Area Drive, P.O. Box 110620, Gainesville, FL 32611, USA (pathos@ufl.edu). [2]Insect Pest Control Laboratory, Joint FAO/IAEA Program of Nuclear Techniques in Food and Agriculture, A-1400 Vienna, Austria [3]Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, NL

17:50 **36 A viral ancestor for the Virus-like particles of the ichneumonid wasp *Venturia canescens***
Apolline PICHON[1], Serge URBACH[3], Jean-Marc AURY[4], Annie BEZIER[2], Véronique JOUAN[1], Marc RAVALLEC[1], François WURMSER[2], Julie GUY[4], Valérie BARBE[4], François COUSSERANS[1], Jeremy GAUTHIER[2], Edith DEMETTRE[3], Vonnick SIBUT[2], Jean-Michel DREZEN[2], Anne-Nathalie VOLKOFF[1]
[1] INRA (UMR 1333), Université de Montpellier 2, "Insect-Microorganisms Diversity, Genomes and Interactions", Place Eugène Bataillon, CC101, 34095 Montpellier Cedex, France. (volkoff@supagro.inra.fr). [2] Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Faculté des Sciences et Techniques, Université F. Rabelais, Parc de Grandmont, 37200, Tours, France. [3] "Functional Proteomics Platform" BioCampus Montpellier, CNRS UMS3426, INSERM US009, Institut de Génomique Fonctionnelle, CNRS UMR5203, INSERM U661, Université de Montpellier 1 et 2, 34094 Montpellier, France. [4] Commissariat à l'Energie Atomique (CEA), Institut de Génomique (IG), "Génoscope", 2, rue Gaston-Crémieux, CP 5706, 91057 Evry, France.

Contributed Papers Monday, 16:30-18:00. Ellwood

Microbial Control 1

Moderator: Matt Thomas

16:30 **37 *Drosophila suzukii*: searching for a microbial control**
Joan Cossentine[1], Dong Xu[2] and Jean-Charles Côté[2]
[1]Pacific Agri-Food Research Centre, 4200 Highway 97, Summerland, BC V0H 1Z0 Canada. [2]Horticulture Research and Development Centre, 430 Gouin Blvd, St-Jean-sur-Richelieu, Québec, Canada, J3B 3E6.
(joan.cossentine@agr.gc.ca)

16:45 **38 Efficacy of a Cuban *Spodoptera frugiperda* MNPV in laboratory assays and preliminary field trials**
Michelle T. Franklin[2], Jorge L. Ayala-Sifontes[1], Amy Huang[2], and Deborah E. Henderson[2] [1]Plant Protection Branch, M. Agri., Sancti Spíritus, Cuba, [2]Institute for Sustainable Horticulture, Kwantlen Polytechnic University, BC Canada (deborah.henderson@kwantlen.ca)

17:00 **39 Effectiveness of dsRNA versus siRNA in RNAi mediated gene knock-down in western corn rootworm (*Diabrotica virgifera virgifera*)**
Chitvan Khajuria[1], Huarong Li[2], Ken Narva[2], Murugesan Rangasamy[2], Blair Siegfried[1] [1]Department of Entomology, University of Nebraska-Lincoln, Entomology Hall, Lincoln, NE 68583, USA. [2]Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268, USA. (ckhajuria2@unl.edu)

17:15 **40 Malaria Mosquitoes Attracted by Fatal Fungus**
Justin George[1], Nina E. Jenkins[1], Simon Blanford[1,2], Matthew B. Thomas[1,2], and Thomas C. Baker[1]
[1]Department of Entomology, Pennsylvania State University, University Park, PA 16802, USA;
[2]Center for Infectious Disease Dynamics, Pennsylvania State University, University Park, PA 16802, USA.

17:30 **41 Studies on effect of spinetoram in *Helicoverpa armigera* (Hübner)**
Lili Zhang, Bingtang Xie, Gemei Liang, Yuyuan Guo
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of

Agricultural Sciences, Beijing 100193, P.R. China. (gmliang@ippcaas.cn)

Contributed Papers — Monday, 16:30-18:15. Haselton

Fungi 2

Moderator: Drauzio Rangel

16:30 **43 STU** **Expression of *Bombyx mori* cecropin A in *Beauveria bassiana* ERL1170 to enhance mycotized mealworms for use as animal feed additives**
Se Jin Lee[1], Jong Cheol Kim[1], Jeong Seon Yu[1], Teak Soo Shin[2] and Jae Su Kim[1] [1]Department of Agricultural Biology, College of Agricultural & Life Sciences, Chonbuk National University, Jeonju 561-756, Republic of Korea. [2]AgroLife Research Institute, Dongbu Farm Hannong Co.,Ltd., Daejeon 305-708, Republic of Korea. (godjin0920@naver.com)

16:45 **44** **Protein and *Metarhizium*: are protein-loving locusts inadvertently increasing their chances of fungal infection?**
Robert I. Graham[1,2], Juliane M. Deacutis[2], Kenneth Wilson[1], Stephen J. Simpson[2] [1]Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK; [2]School of Biological Sciences and Charles Perkins Centre, University of Sydney, NSW 2006, Australia (r.graham@lancaster.ac.uk)

17:00 **45** **Effect of physiographic and climatic conditions on development of epizootics by *Entomophaga maimaiga* in outbreak gypsy moth populations**
Ann E. Hajek[1], Patrick Tobin[2], Kyle Haynes[3]
[1]Department of Entomology. Cornell University, Ithaca, New York 14853-2601, USA. [2]USDA Forest Service, Northern Research Station, Morgantown, West Virginia 26505, USA. [3]Department of Environmental Sciences, Blandy Experimental Farm, University of Virginia, Boyce, Virginia 22620, USA.

17:15 **46** **Bumblebee venom serine protease increases fungal insecticidal virulence by inducing insect melanization**
Jae Su Kim[1], Jae Young Choi[2], Byung Rae Jin[3], Margaret Skinner[4], Bruce L. Parker[4] , Se Jin Lee[1] and Yeon Ho Je[2]
[1]**Department of Agricultural Biology, College of Agricultural & Life Sciences, Chonbuk National University, Jeonju 561-756, Korea.** [2]Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Seoul 151-742, Korea. [3]Department of Applied Biology, College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea. [4]Entomology Research Laboratory, University of Vermont, Burlington 05405, USA. (jskim10@jbnu.ac.kr)

17:30 **47** **Using *Drosophila* as a model system for analyzing insect-fungal interactions**
Hsiao-ling Lu and Raymond St Leger
Department of Entomology, University of Maryland, 4112 Plant Sciences Building, College Park, MD, 20742 (hllu@umd.edu)

17:45 **48** **Characterization of a novel secreted insect toxic protein (Sit1) from the entomopathogenic fungus *Metarhizium anisopliae***
Almudena Ortiz-Urquiza[1,2], Enrique Quesada-Moraga[1], and Nemat O. Keyhani[2] [1]Department of Agricultural and Forestry Sciences, ETSIAM, University of Cordoba. Campus de Rabanales, Cordoba 14071. España (Spain).
[2]Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611 (almudenaortiz@ufl.edu)

18:00 **49** **Role of two Loss-of-aflatoxin expression (LaeA)-like putative methyltransferases in *Beauveria bassiana* development and virulence**
Yuqi Qin[1,2], Yinbo Qu[1], and Nemat O. Keyhani[2]
[1] National Glycoengineering Research Center, Shandong University, 27, Shanda South Road, Jinan, Shandong 250100, PR China [2]Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611 (qinyuqi@sdu.edu.cn)

SIP Division Business Meetings: 20:00 – 21:30

Microbial Control — Ellwood

Diseases of Beneficial Invertebrates & Discussion: How can you tell a live from a dead zebra mussel (no joke!)? — Haselton

Microsporidia & EUPATH — Fountainview

TUESDAY - 13 August

08:00–17:00 Registration **Lobby**

07:00–08:00 **BREAKFAST** Grand Station III-V

Symposium (Disease Beneficial Invertebrates) Tuesday, 08:00-10:00 Grand Station I-II

Pathogens to control populations of invasive aquatic invertebrates

Organizers/Moderators: Grant Stentiford & Stefan Jaronski

08:00 **50 Challenges in microbial control of invasive mosquitoes and lessons learned**
James J. Becnel
Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL 32608
(James.Becnel@ars.usda.gov)

08:20 **51 Can aquatic invasive invertebrates be controlled by re-introduction to their native pathogens?**
Stentiford, G.D.* and Stebbing, P.D.
European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, The Nothe, Barrack Road, Weymouth, Dorset, DT4 8UB, United Kingdom
*E-mail: grant.stentiford@cefas.co.uk

08:40 **52 Exploration of potential microbial control agents for the invasive crayfish, *Orconectes virilis***
Elizabeth W. Davidson[1], Jennifer Snyder[2], Donald Lightner[3], Gregory Ruthig[4] Julie Lucas[1] and Joel Gilley[1]
[1]School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501; [2]Oregon State University, 104 Nash Hall, Corvallis, OR 97331; [3] Department of Veterinary Science and Microbiology, University of Arizona, 1117 East Lowell Street, Tucson, AZ 85721, [4]Grinnell College, Grinnell, Iowa 50112
(e.davidson@asu.edu)

09:00 **53 Microbial control of an invasive aquatic mollusc, the zebra mussel – The quest from the researcher's perspective**
Daniel P. Molloy[1,2]
1. Department of Biological Sciences, State University of New York, Albany, NY 12222 USA.
2. New York State Museum, Albany, NY USA.
dmolloy@albany.edu

09:20 **54 Development of a Microbial Control for Invasive Quagga and Zebra Mussels**
Carolyn Link, MSc, Sarahann Rackl, Ph.D., P.E., Pam Marrone, Ph.D. Marrone Bio Innovations, Inc., 2121 Second Street, Suite B-107, Davis, CA 95618
(CLink@marronebio.com)

Contributed Papers Tuesday, 08:00-10:00. Ellwood

Fungi 3

Moderators: Ingeborg Klingen & Helen Hesketh

08:00 **55 STU Investigating Asian longhorned beetle immunity following maternal immunepriming with a fungal pathogen**
Joanna J. Fisher and Ann E. Hajek
Department of Entomology, Cornell University, Ithaca NY 14853-2601, USA. (jjf236@cornell.edu)

08:15 **56 Occurrence of *Metarhizium anisopliae* in an organic, rotational no-till cropping system**
Mary E. Barbercheck and Christina Mullen
Department of Entomology, Penn State University, 501 Agricultural Sciences & Industries Building, University Park, PA, 16802, USA. (meb34@psu.edu)

08:30 **57 Laboratory and greenhouse evaluation of a new entomopathogenic strain of *Beauveria bassiana* for control of the onion thrips *Thrips tabaci***
Yulin Gao[1], Zhongren Lei[1], Jing Wang[1], Shengyong Wu[1], Xuenong Xu[1], Guy Smagghe[2], and Stuart Reitz[3]
[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R. China.
[2]Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium.
[3]Malheur County Extension, Oregon State University, 710 SW 5th Ave, Ontario, OR 97914, USA. (ylgao@ippcaas.cn; zrlei@ippcaas.cn)

08:45 **58 Investigating Metarhizium brunneum F52 microsclerotia applied as a hydromulch for the potential biological control Asian long horned beetle, Anoplophora glabripennis**
Tarryn Anne Goble[1], Ann E Hajek[1],Sana Gardescu[1] and Mark Ja
[1]Department of Entomology, Cornell University, Ithaca, New Y 14853-2601, USA. [2] USDA ARS NCAUR, Peoria, Illinois 6160 USA. (tazgoble@gmail.com)

09:00 **59 *Beauveria bassiana* as a potential agents for emerald ash borer management: Tracking tool to monitor a post-release isolate.**
George Kyei-Poku and Shajahan Johny
Canadian Forestry Service, Great Lakes Forestry Centre, Natural Resources Canada, 1219 Queen Street East, Sault Ste. Marie, Ontario, Canada P6A 2E5. (jshajaha@uoguelph.ca)

09:15 **60 The effect of *Neozygites floridana* killed *Tetranychus urticae* females on sexual behavior of *T. urticae* males**
Ingeborg Klingen[1], Upendra Raj Bhattarai[1,2], Karin Westrum[1], Geir Kjølberg Knudsen[1], Nina Trandem[1]
[1]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Aas, Norway. [2]Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, PO Box 5003, 1432 Aas, Norway.
(ingeborg.klingen@bioforsk.no)

09:30 **61 STU *Neozygites osornensis* sp. nov., a new fungal species causing mortality to the cypress aphid *Cinara cupressi* in Chile**
Cristian Montalva[1], Marek Barta[2], Eladio Rojas[3], Nolberto Arísmendi[4], Dolly Lanfranco[5] Eduardo Valenzuela[6]
[1]Universidad Austral de Chile, Facultad de Ciencias Forestales y Recursos Naturales, Escuela de Graduados, Casilla 567, Valdivia, Chile. (cristian.montalva@alumnos.uach.cl).
[2] Department of Dendrobiology, Mlyňany Arboretum SAS, Vieska nad Žitavou 178, Slepčany 95152, Slovakia.
[3] Laboratorio Regional, Servicio Agrícola y Ganadero, Ruta a Puerto Octay U-55-V, Calle de Servicio, Osorno, Chile.
[4] Universidad Austral de Chile, Facultad de Ciencias Agrarias, Escuela de Graduados, Casilla 567, Valdivia, Chile.
[5]Universidad Austral de Chile Facultad de Ciencias Forestales y Recursos Naturales. Instituto de Silvicultura. Valdivia, Chile.
[6] Universidad Austral de Chile, Facultad de Ciencias, Instituto de Microbiología, Casilla 167, Valdivia, Chile.

09:45 **62 Microbial control of the Asian ambrosia beetle *Xylosandrus germanus***
John Vandenberg[1], Louela Castrillo[2] and Michael Griggs[1]
[1]USDA ARS Robert W. Holley Center for Agriculture and Healthy, 538 Tower Road, Ithaca NY 14853. [2]Department of

Entomology, Cornell University, Ithaca NY 14853. (john.vandenberg@ars.usda.gov)

Contributed Papers — Tuesday, 08:00-09:45. Haselton

Bacteria 2

Moderators: Hyun Woo & Blair Siegfried

08:00 **63 Novel protein production system using a peptide-tag derived from *Bacillus thuringiensis* mosquitocidal Cry4Aa toxin.**
Tohru Hayakawa[1], Shinya Sato[2], Shigehisa Iwamoto[2], Shigeo Sudo[2], Mohammad, T. H. Howlader[1], Hiroshi Sakai[1]
[1]Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-Naka, Okayama 700-8530, Japan. [2] Department of Bioscience, Japan Lamb Co. Ltd, Rm 504 SKIP-city, SAITEC, 3-12-18 Kamiaoki, Kawaguchi, Saitama 333-0844, Japan. (hayaka-t@cc.okayama-u.ac.jp)

08:15 **64 The novel gene discovery of *Bacillus thuringiensis* in northeast region, China**
Haitao Li , Rongmei Liu , JiGuo Gao
Northeast Agricultural University, HarBin 150030, People's Republic of China

08:30 **65 Regulation and roles of "host iron" acquisition systems in *Bacillus cereus & B. thuringiensis* during infection**
Diego Segond[1], Elise Abi Khalil[1], Christophe Buisson[1], Ahmed Yakoubi[1], Amani Farhat[1], Mireille Kallassy and Christina Nielsen-LeRoux [1] [1]INRA,UMR 1319 Micalis-AgroparisTech, La Minière, 78280 Guyancourt, France. [2]Laboratory of Biotechnology, Saint-Joseph University, Beyrouth, Lebanon.(Christina.nielsen@jouy.inra.fr)

08:45 **66 Molecular Genetic Basis for Engineering Small to Large Crystals of Cyt1Aa**
Hyun-Woo Park[1,2], Robert H. Hice[1] and Brian A. Federici[1,3]
[1]Department of Entomology, University of California, Riverside, Riverside, CA 92521, USA. [2]Department of Natural and Mathematical Sciences, California Baptist University, Riverside, CA 92504, USA. [3]Interdepartmental Graduate Programs in Genetics, Genomics & Bioinformatics and Cell, Molecular & Developmental Biology, University of California, Riverside, Riverside, CA 92521, USA. (hwpark@ucr.edu)

09:00 **67 The toxins and their synergy in Bacillus thuringiensis strain HBF-18**
Yang Bi [1], Yanrui Zhang [1], Changlong Shu [1], Qinglei Wang [2], Lili Geng [1], Lixin Du [3], Fuping Song [1], Jie Zhang [1]
[1] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, China. (jzhang@ippcaas.cn) [2] Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Baoding, 071000, China. [3] Cangzhou Academy of Agricultural and Forestry Sciences, Cangzhou, 061001, China.

09:15 **68 Screening of *cry, vip* and *cyt* genes in *Bacillus thuringiensis* strains collected from the Amazon biome in Brazil.**
[2]André Mourão; [2]Arthur Torres; [1]Rosane Silva; ; [3]Fernando Valicente [1]Federal University of Lavras [2]Federal University of São João Del Rei [3]Embrapa Maize and Sorghum Research Station (fernando.valicente@embrapa.br)

09:30 **69 *cry, vip* and *cyt* genes occurrence in *Bacillus thuringiensis* strains isolated from the Cerrado region in Western Central in Brazil.**
[2]Arthur Torres; [2]André Mourão; [1]Rosane Silva; ; [3]Fernando Valicente [1]Federal University of Lavras [2]Federal University of São João Del Rei [3]Embrapa Maize and Sorghum Research Station (fernando.valicente@embrapa.br)

Contributed Papers — Tuesday, 08:00-10:00. Fountainview.

Nematodes 2

Moderators: David Shapiro-Ilan & Luis Leite

08:00 **70 The response of *Caenorhabditis elegans* to bacteria from its natural environment**
Amanda C. Wollenberg[1], Anna-Maria F. Alves[1], Marie-Anne Félix[2] and Javier E. Irazoqui[1]
[1]Program in Developmental Immunology, Massachusetts General Hospital, 55 Fruit St. GRJ 1402, Boston, MA 02114, USA. [2]Institut de Biologie de L'Ecole Normale Supérieure, 46 Rue d'Ulm, 75005 Paris, France. (acwollenberg@gmail.com)

08:15 **71 Biological control potential of five Turkish entomopathogenic nematode isolates against the lawn caterpillar, *Spodoptera cilium***
Baris GULCU[1], Derya ASICI[2], Mehmet KARAGOZ[3], Selcuk HAZIR[2]
Duzce University, Faculty of Arts and Sciences, Department of Biology, 81620, Turkey[1]
Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, 09100, Turkey[2] Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, 09100, Turkey[3] (barisgulcu@duzce.edu.tr)

08:30 **72 Does Combining Entomopathogenic Nematode Species Enhance Control of Curculio elephans (Coleoptera, Curculionidae) Overwintering Larvae?**
[1]Selcuk Hazir, [2]Sevdiye Demir, [2]Mehmet Karagoz, [3]Harry K. Kaya [1]Adnan Menderes University, Faculty of Arts and Sciences, Department of Biology, Aydin-Turkey. [2]Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, Aydin-Turkey. [3]University of California, Nematology Department, One Shields Ave., Davis, CA, USA.

08:45 **73 STU Nematicidal activity of Crude Extracts of the Entomopathogenic Bacterium, *Photorhabdus l. sonorensis* on the Root-knot Nematode (*Meloydogyne incognita*) and the Stem Gall Nematode (*Anguina pacificae*)**
Rousel A. Orozco, S. Patricia Stock.
Department of Entomology, University of Arizona, Tucson AZ, 85721-0036, USA. (rouselo@email.arizona.edu)

09:00 **74 Potential of native nematodes as pest snail control agents in Australia**
Aisuo Wang[1, 2] and Gavin Ash[1, 2]
[1]School of Agricultural and Wine Sciences, Charles Sturt University, Boorooma Street Wagga Wagga NSW 2678 Australia. [2]Graham Centre for Agricultural Innovation, Pugsley Place Wagga Wagga NSW 2650 Australia (awang@csu.edu.au)

10:00–10:30 **BREAK** Grand Station I-II

Symposium (Microbial Control) — Tuesday, 10:30-11:30. Grand Station I-II.

Duking it out – interactions between introduced microbial pest control agents and indigenous microflora

Organizers/Moderators: Stefan Jaronski & Pesco Avery

10:30 **76 Insect Pathogen-Indigenous Microbe Interactions in the Rhizosphere**
Cindy Fuller and Stefan Jaronski

Henderson State University, Arkadelphia, AR, and United States Department of Agriculture, Northern Plains Research Laboratory, Sidney, MT (fuller@hsu.edu)

11:00 **77 Determining the fate of introduced *Beauveria bassiana* GHA in agricultural fields and its impact on conspecific indigenous populations**
Louela A. Castrillo
Department of Entomology, Cornell University, Ithaca, NY 14853, USA (lac48@cornell.edu)

Contributed Papers — Tuesday, 10:30-12:30. Ellwood

Viruses 1

Moderator: Adly M.M. Abd-Alla & Eric J. Haas-Stapleton

10:30 **78 Managing hytrosavirus infections in *Glossina pallidipes* colonies: Feeding regime affects the prevalence of salivary gland hypertrophy syndrome**
Adly M. M. Abd-Alla[a*#], Henry M. Kariithi[a, b#], Abdul Hasim Mohamed[a], Edgardo Lapiz[a], Andrew G. Parker[a], and Marc J.B. Vreysen[a]
[a] Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, A-1400 Vienna, Austria, [b] Laboratory of Virology, Wageningen University, 6708 PB Wageningen, The Netherlands.

10:45 **79 *Microplitis demolitor* bracovirus (MdBV) persists in a semi-permissive host *Trichoplusia ni***
Kavita Bitra[1] and Michael R Strand[1]
Department of Entomology, University of Georgia, 120 Cedar street, Athens, Georgia - 30602, USA. (kbitra@uga.edu)

11:00 **80 A Survey of Single-stranded RNA Viruses in the Tarnished Plant Bug, *Lygus lineolaris***
Omaththage P. Perera, Gordon L. Snodgrass, Clint Allen, and Randall G. Luttrell
Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS, USA (Op.perera@ars.usda.gov)

11:15 **81 *Wolbachia* increases host susceptibility to *Spodoptera exempta* nucleopolyhedrovirus**
Robert I. Graham[1], David Grzywacz[2], Wilfred L. Mushobozi[3], Kenneth Wilson[1] [1]Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK; [2]Natural Resources Institute, University of Greenwich at Medway, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK; [3]EcoAgriConsultancy Services Ltd, Nairobi Road, Kwa Idd, PO Box 15040 Arusha, Tanzania. (r.graham@lancaster.ac.uk)

11:30 **82 STU Possible correlation between genetic diversity and viral activity in different Polish LdMNPV isolates.**
Martyna Krejmer[1],Lukasz Rabalski[1], Iwona Skrzecz[2], Boguslaw Szewczyk[1]
[1]Department of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology of the University of Gdansk and Medical University of Gdansk, ul. Kladki 24, 80-822 Gdansk, Poland (martyna.krejmer@biotech.ug.edu.pl). [2]Forest Research Institute, ul. Braci Lesnej 3, 05-090 Raszyn, Poland.

11:45 **83 STU A novel pea aphid antiviral defense strategy**
Diveena Vijayendran, Sijun Liu and Bryony C. Bonning
Department of Entomology, Iowa State University, IA 50014, USA. (diveena@iastate.edu)

12:00 **84 Isolation and characterization of host ranges extended baculoviruses through co-infection approach in insect cells**
Tzong-Yuan Wu
Department of Bioscience Technology,
Chung Yuan Christian University, Chungli 32023, Taiwan

12:15 **85 Characterization of baculoviruses from the Martignoni collection**
George F. Rohrmann Dept. of Microbiology Oregon State University Corvallis, OR 97331-3804
USA (rohrmanng@orst.edu)

Contributed Papers — Tuesday, 10:30-12:00. Haselton

Diseases of Beneficial Invertebrates 1

Moderator: Lena Poppinga & Elka Genersch

10:30 **86 Dynamics of the presence of IAPV in CCD colonies**
Chunsheng Hou[1], Hadassah Rivkin[1], Yossi Slabezki[2] and Nor Chejanovsky[1]
[1]Entomology Department, Institute of Plant Protection, The Volcani Center, Israel. [2]Beekeeping Division, Extension Service, Israeli Ministry of Agriculture.
(ninar@volcani.agri.gov.il)

10:45 **87 STU Different strategies of *Paenibacillus larvae* to evade the immune response of honey bee larvae**
Gillian Hertlein, Lena Poppinga, Eva Garcia-Gonzalez, Anne Fünfhaus, Kati Hedtke, Elke Genersch
Dep. of Diagnostic and Molecular Biology, Institute for Bee Research Hohen Neuendorf, Friedrich-Engels-Str. 32, D-16540 Hohen Neuendorf, Germany (elke.genersch@rz.hu-berlin.de)

11:00 **88 Survey of chalkbrood fungi infecting alfalfa leafcutting bees in U.S. alfalfa seed fields**
James, Rosalind R., Klinger, Ellen, G., Pitts-Singer, Theresa L.
USDA-ARS Pollinating Insects Research Unit, Dept. Biology, Utah State Univ., Logan, UT 84322
(Rosalind.James@ars.usda.gov)

11:15 **89 STU Pathogenicity of mixed infections of *Ascosphaera* in solitary and social bees**
Klinger, Ellen G. [1, 2], Vojvodic, Svjetlana[3], and James, Rosalind R.[2]
[1]Utah State University; 5310 Old Main Hill; Logan, UT; 84341
[2] USDA-ARS Pollinating Insect Research Unit; 1410 North 800 East; Logan, UT; 84341
[3] Center for Insect Science, University of Arizona; 1041 E. Lowell St.; Tucson, AZ 85721
(ellen.klinger@ars.usda.gov)

11:30 **90 Histopathology of infectious diseases of the honey bee (*Apis mellifera*)**
Lena Poppinga[1], Heike Aupperle[2], Elke Genersch[1]
[1] Institute for Bee Research, Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany
[2] Laboklin GmbH & Co KG, Bad Kissingen, Germany
(elke.genersch@rz.hu-berlin.de)

11:45 **91 Are pesticides affecting pathogen levels and immunity in honey bees?**
Brenna E. Traver[1], Nels G. Johnson[2], Katelyn M. Catalfamo[3], Haley K. Feazel-Orr[3], Troy D. Anderson[1], and Richard D. Fell[1]
[1]Department of Entomology, Virginia Tech, 216 Price Hall, Blacksburg, VA 24061, USA. [2]Department of Biology, Colorado State University, Campus Delivery 1878, Fort Collins, CO 80523, USA, [3]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA. (traverb@vt.edu)

Workshop (Fungi and Microbial Control) — Tuesday, 11:30-13:00. Fountainview

What's the name of my fungus?

Organizer: Nicolai vitt Meyling

11:30 **92 Current status of phylogenetic reclassifications: Here**

today and gone tomorrow
Richard A. Humber
USDA-ARS Biological IPM Research, RW Holley Center for Agriculture and Health, 538 Tower Road, Ithaca, NY 14853, USA. (richard.humber@ars.usda.gov)
[3] Center for Insect Science, University of Arizona; 1041 E. Lowell St.; Tucson, AZ 85721 (ellen.klinger@ars.usda.gov)

12:00 **93 Field to Phylogeny: Molecular identification of *Beauveria* and *Metarhizium* species**
Ryan M. Kepler[1] and Stephen A. Rehner[1]
[1]Systematic Mycology and Microbiology Laboratory, USDA-ARS, Bldg 010A, Beltsville, MD 20705, USA (Ryan.Kepler@ars.usda.gov)

12:30 **94 Molecular tools for strain detection and population genetic analyses of *Metarhizium* and *Beauveria* spp.**
Jürg Enkerli, Andy Lutz, Franco Widmer
Molecular Ecology, Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, CH 8046 Zürich, Switzerland (Juerg.enkerli@agroscope.admin.ch)

12:30–16:00 **LUNCH & EXCURSION**

13:00 & 13:15 **Buses leave Sheraton for museum**

Not participating in 5K:

15:45 & 16:00 **Buses leave museum for Sheraton**

Participating in 5K:

15:30 **Bus leaves museum for 5K run/walk**

15:30 **Bus leaves Sheraton for 5K run/walk**

16:00–17:30 **5K RUN/WALK**

18:00 **BOAT BOARDING FOR BBQ**

18:30 **CAST OFF**

18:00–22:00 **BBQ**

WEDNESDAY - 14 August

08:00–17:00 Registration **Lobby**

07:00–08:30 **BREAKFAST** Grand Station III-V

Symposium (Fungi) Wednesday, 8:30–10:30. Grand Station I-II

Forty years of ARSEF: success of an essential germplasm resource

Organizers/Moderators: John Vandenberg

08:30 **95 Spreading culture: From a refrigerator to the whole world**
Richard A. Humber
USDA-ARS Biological IPM Research, RW Holley Center for Agriculture and Health, 538 Tower Road, Ithaca, NY 14853, USA. (richard.humber@ars.usda.gov)

08:50 **96 Importance of exploration to the success of ARSEF, an essential germplasm resource for entomopathogenic-fungi research**
Donald W. Roberts
Department of Biology, Utah State University, Logan, UT 84322-5305 USA (donald.roberts@usu.edu)

09:10 **97 Genome-driven insights into the phylogeny, population biology and molecular ecology of *Beauveria* and *Metarhizium***
Stephen A. Rehner[1] and Ryan M. Kepler[1]
[1]Systematic Mycology and Microbiology Laboratory, USDA-ARS, Bldg 010A, Beltsville, MD 20705, USA (stephen.rehner@ars.usda.gov)

09:30 **98 Studies on host-pathogen interactions using isolates from the ARSEF collection**.
Raymond St. Leger
Department of Entomology, 4112 Plant Sciences Building, University of Maryland, College Park, MD 20742, stleger@umd.edu

09:50 **99 How does the ARSEF collection contribute to studies on ecology of insect pathogenic fungi?**
Jørgen Eilenberg
Department of Plant and Environmental Sciences, University of Copenhagen,Thorvaldsensvej 40 DK 1871, Frederiksberg C., Denmark, jei@life.ku.dk

10:10 **100 The ARSEF collection and 40 years of microbial biocontrol with entomopathogenic fungi**
Stephen P. Wraight
USDA-ARS Robert W. Holley Center for Agriculture and Health, Biological Integrated Pest Management Research Unit, Ithaca, NY 14853 USA. (steve.wraight@ars.usda.gov)

Symposium (Microsoridia) Wednesday, 8:30–10:30. Ellwood

Graduate student studies of microsporidia and other protists

Organizer/Moderator: Carlos Lange & Susan Bjørnson

08:30 **101 STU Genetic architecture underlying variation in *Caenorhabditis elegans* host resistance to natural microsporidia infection**
Keir M. Balla[1], Erik C. Andersen[2], Leonid Kruglyak[3], Emily R. Troemel[1] [1]Division of Biological Sciences, University of California San Diego [2]Molecular Biosciences, Northwestern University [3]Lewis-Sigler Institute for Integrative Genomics, Princeton (kballa@ucsd.edu)

08:50 **102 STU Microsporidia and the two-spotted lady beetle *Adalia bipunctata* L.**
Thomas Steele; Susan Bjørnson
Biology Department, Saint Mary's University, 923 Robie Street, Halifax, NS B3H 3C3 Canada.
steelem4@hotmail.com

09:10 **103 STU Immune response of *Lymantria dispar* to naturally occurring intracellular pathogens**
Gwyn L. Puckett[1], Leellen F. Solter[2], Marianne Alleyne[1], [3]Peter M. Yau, [3]Brian S. Imai
[1]Department of Entomology, University of Illinois (puckett4@illinois.edu); [2]Illinois Natural History Survey, University of Illinois; [3]Biotechnology Center, University of Illinois Urbana-Champaign

09:30 **104 STU The potential use of a host specific biological treatment against locusts**
Schoeters Floris[1], Boerjan Bart[1], Lavigne Rob[2], Schoofs Liliane[1]
[1]Functional Genomics and Proteomics Group, Department of Biology, KU Leuven-University of Leuven, Belgium.
[2]Division of Gene Technology, Department of Bioengineering, KU Leuven-University of Leuven, Belgium.
(floris.schoeters@student.kuleuven.be)

09:50 **105 STU Studying the molecular and cellular evolution of intranuclear microsporidia in crabs**
Dominic Wiredu Boakye, Bryony Williams and Grant Stentiford
College of Life and Environmental Sciences. University of Exeter, Geoffrey Pope. Stocker Road, Exeter. EX4 4QD. (DW347@exeter.ac.uk)

Contributed Papers Wednesday, 8:30-10:30. Haselton

Viruses 2

Moderator: Zhihong Hu & David A. Theilman

08:30 **106 A potential role for effector caspases CASPS18 and CASPS19 in midgut escape of Sindbis virus in *Aedes aegypti***
Ning Huang, A. Lorena Passarelli, and Rollie J. Clem
Division of Biology, Kansas State University, Manhattan, KS 66503 (rclem@ksu.edu)

08:45 **107 Strong selective pressure against a recombinant Sindbis virus that induces apoptosis in the midgut of the mosquito *Aedes aegypti***
Katelyn O'Neill and Rollie J. Clem
Division of Biology, Kansas State University, Manhattan, KS (rclem@ksu.edu)

09:00 **108 Inactivation of the budded virus of *Autographa californica* M nucleopolyhedrovirus by gloverin**
Daniela A. Moreno-Habel[1], Ivan M. Biglang-awa[2], Paul M. M. Weers[2], and Eric J. Haas-Stapleton[1]
Department of Biological Sciences[1] or Chemistry and Biochemistry[2], California State University Long Beach, 1250 Bellflower Blvd, Long Beach, CA 90840, USA. (Eric.Haas-Stapleton@csulb.edu)

09:15 **109 Genomic adaptation in the bracovirus of *Cotesia sesamiae* identified by targeted resequencing.**
Jeremy Gauthier[1]; Philippe Gayral[1]; Bruno Leru[2], Severine Jancek[1]; Stéphane Dupas[3]; Laure Kaiser[3], Gabor Gyapay[4], Elisabeth A Herniou[1]
[1]Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS

6035, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Avenue Monge - Parc Grandmont 37200 Tours France; [2]ICIPE, IRD UR72, Nairobi, Kenya; [3]CNRS UR9034, IRD UR72, Université Paris-Sud, Gif-sur-Yvette, France ; [4]UMR8030 Genoscope (CEA) Evry, France (elisabeth.herniou@univ-tours.fr)

09:30 **110** **Functional comparison of the Group I and Group II alphabaculoviruses, *Autographa californica* multiple nucleopolyhedrovirus and *Mamestra configurata* nucleopolyhedrovirus, replication genes *DNA polymerase* and *lef1***
Ajay B. Maghodia[1], Minggang Fang[1], Martin A. Erlandson[2] and David A. Theilmann[1]
[1]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Box 5000, Summerland BC, Canada V0H 1Z0. [2]Saskatoon Research Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2 (david.theilmann@agr.gc.ca)

09:45 **111 STU** **Baculovirus IE2 Forms Novel Visible Nuclear Cage-like Structure as Strong Transcriptional center**
Hsuan Tung[1,2,3] and Yu-Chan Chao[1,2]
[1]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan [2]Molecular and Biological Agricultural Sciences Program, Taiwan International Graduate Program, Academia Sinica, Taipei, Taiwan. [3]Graduate Institute of Biotechnology, National Chung-Hsing University, Taichung, Taiwan. (hsuansandy@gmail.com)

10:00 **112** **Baculovirus photolyases and biological rhythm**
Magdalena A. Biernat[1,2], Ines Chaves[2], Just M. Vlak[1], Gijsbertus T.J. van der Horst[2], Monique M. van Oers[1]
[1]Laboratory of Virology, Wageningen University, Wageningen, the Netherlands, [2]Department of Genetics, Section Chronobiology and Health, Erasmus University Medical Center, Rotterdam, the Netherlands (monique.vanoers@wur.nl)

10:15 **113** **The baculovirus core gene *ac83* is required for nucleocapsid assembly and *per os* infectivity of *Autographa californica nucleopolyhedrovirus***
Shimao Zhu, Wei Wang, Yan Wang, Meijin Yuan and Kai Yang
State Key Laboratory of Biocontrol, Sun Yat-sen University, 517 Northern Building of School of Life Sciences, Southern Campus, Guangzhou 510275, China. (yangkai@mail.sysu.edu.cn)

10:30–11:00 **BREAK** Grand Station III-V

Wednesday, 10:30-12:00. Grand Station III-V
POSTER SESSION

12:00–13:30 **LUNCH** On your own

Wednesday, 12:00-13:30. Haselton
STUDENT WORKSHOP

Wednesday, 12:00-13:30. Ellwood
JIP EDITORIAL

Symposium (Microbial Control & Nematodes) Wednesday, 13:30-15:30. Grand Station I-II
Trait stability and improvement

Organizers: Stefan Jeronski & David Shapiro-Illan

13:30 **114** **Trait stability and improvement of bacterial insecticides**
Brian A. Federici, Department of Entomology and Interdepartmental Graduate Programs in Microbiology, Genetics, and Molecular Biology, University of California, Riverside, Riverside, California 92521 U.S.A. (brian.federici@ucr.edu)

14:00 **115** **Trait Stability Among the Entomopathogenic Fungi**
Stefan. T. Jaronski
U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Agriculture Laboratory, Sidney MT USA (stefan.jaronski@ars.usda.gov)

14:30 **116** **Trait stability and improvement in entomopathogenic viruses: lessons learnt from baculoviruses**
Johannes A. Jehle
Institute for Biological Control, Federal Research Centre for Cultivated Plants, Julius Kühn Institute (JKI), Heinrichstr. 243, 64287 Darmstadt, Germany; E-mail: Johannes.Jehle@jki.bund.de

15:00 **117** **Trait stability and improvement in entomopathogenic nematodes**
David I. Shapiro-Ilan[1], Byron J. Adams[2], Selcuk Hazir[3], and Ralf-Udo Ehlers[4]
[1]USDA-ARS, SEFTNRL, 21 Dunbar Road, Byron, GA 31008 USA. [2]Microbiology & Molecular Biology Department, and Evolutionary Ecology Laboratories, Brigham Young University, Provo, UT 84602 USA. [3]Adnan Menderes University, Faculty of Arts and Science, Department of Biology, 09010 Aydin, Turkey. [4]e-nema, Gesellschaft für Biotechnologie, und biologischen Pflanzenschutz mbH, Klausdorfer Str. 28-36, 24223 Schwentinental/Germany. (David.Shapiro@ars.uda.gov)

Contributed Papers Wednesday, 13:30-15:15. Ellwood
Diseases of beneficial invertebrates 2
Moderator: Kelly Bateman

13:30 **118** **Planning a "Needle in a Haystack" project – The search for the alternate, obligate host(s) of the zebra mussel parasite *Haplosporidium raabei***
Daniel P. Molloy[1,2]
1. Department of Biological Sciences, State University of New York, Albany, NY 12222 USA.
2. New York State Museum, Albany, NY USA.
dmolloy@albany.edu

13:45 **119** **Characterizing infection responses in the Pacific white shrimp, *Litopenaeus vannamei***
[1,2]Duan Loy, [1]Sijun Liu, [1]Lyric Bartholomay
Departments of [1,2]Entomology and [2]Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames IA 50011 (lyricb@iastate.edu)

14:00 **120 STU** **Does the Common shore crab (*Carcinus maenas*) show resistance to White Spot Disease?**
Kelly S. Bateman, Michelle Pond, David Stone and Grant D. Stentiford
European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK. Address for correspondence: kelly.bateman@cefas.co.uk

14:15 **121** ***Apicystis bombi*, a protozoan parasite of bumblebees, acts as an emergent infectious disease**

Jafar Maharramov[1], Ivan Meeus[1], Kevin Maebe[1], Marina Arbetman[2], Carolina Morales[2], Peter Graystock[3], William O. H. Hughes[4], Santiago Plischuk[5], Carlos E. Lange[5], Dirk C. De Graaf[6], Nelson Zapata[7], Jose Javier Perez de la Rosa[8], Tomás E. Murray[9,10], Mark Brown[11] and Guy Smagghe[1]
[1]Deptartment of Crop protection, Ghent University, Coupure 653, 9000 Ghent, Belgium. [2]Laboratorio Ecotono, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue INIBIOMA (Conicet), Quintral 1250, 8400 Bariloche, Rı´o Negro, Argentina. [3]School of Biology, University of Leeds, Leeds, LS2 9JT, UK. [4]School of Life Sciences, University of Sussex, Brighton, BN1 9QG, UK. [5]Centro de Estudios Parasitológicos y de Vectores CEPAVE (CCTLP CONICET-UNLP-CIC) La Plata,Buenos Aires, Argentina. [6]Laboratory of Zoophysiology, Department of Physiology, Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium. [7]Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Concepción, Avenida Vicente Méndez 595, Chillán, Chile. [8]Centro Nacional de Servicios de Constatacion en Salud Animal (CENAPA), km 11.5 Carretera Federal Cuernavaca-Cuautla No.8534, Jiutepec, Morelos, Mexico. [9]Teagasc, Oak Park Research Centre, Oak Park, Carlow, Co. Carlow, Ireland.[10]Martin-Luther University Halle-Wittenberg, Institute for Biology, Department of Zoology, D-06120 Halle (Saale), Germany. [11]School of Biological Sciences, Royal Holloway, University of London, Egham, TW20 0EX, United Kingdom. (Jafar.Maharramov@ugent.be)

14:30 **122** **Dicistroviruses in bumblebees: pathology and eradication**
Ivan Meeus[1], Jinzhi Niu[1], Joachim R. de Miranda[2], Felix Wäckers[2], Guy Smagghe[1]
[1]Department of Crop Protection, Ghent University, Coupure 653, 9000 Ghent, Belgium.
[2]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[3]Biobest, Ilse Velden 18, 2260 Westerlo, Belgium
(ivan.meeus@UGent.be)

14:45 **123 STU** **The interaction between IAPV and bumblebee's RNAi and JAK-STAT pathways based on qPCR analysis**
Jinzhi Niu[1], Ivan Meeus[1], Kaat Cappelle[1], Joachim R. de Miranda[2], Guy Smagghe[1]
(Jinzhi.niu@ugent.be)
[1]Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium
[2]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

15:00 **124** **An investigation of oomycetes infecting rotifers in Brooktrout lake, NY – Lethal parasites that produce outgrowths with distinctive morphologies but of unknown function**
Daniel P. Molloy[1,2]
1. Department of Biological Sciences, State University of New York, Albany, NY 12222 USA.
2. New York State Museum, Albany, NY USA.
dmolloy@albany.edu

Contributed Papers — Wednesday, 13:30-15:00. Haselton

Microsporidia 1

Moderator: Susan Bjørnson

13:30 **125** **A new isolate of *Nosema* sp. (Microsporidia, Nosematidae) from *Malacosoma disstria* (Lepidoptera, Lasiocampidae).**
George Kyei-Poku, Shajahan Johny, Debbie Gauthier and Yuehong Liu
Canadian Forestry Service, Great Lakes Forestry Centre, Natural Resources Canada, 1219 Queen Street East, Sault Ste. Marie, Ontario, Canada P6A 2E5. (gkyeipok@nrcan.gc.ca)

13:45 **127** **Comparative genomics of marine microsporidia**
Bryony Williams
Biosciences, Geoffrey Pope Building, Stocker Road, University of Exeter, Devon, UK, EX4 4EU.
b.a.p.williams@exeter.ac.uk

14:00 **128** **Microsporidia play the "bad guys" in a biological control program**
Huang, W-F.[1], Solter, L.F.[1], Onken, B.[2], Havill, N.P.[3]
[1]Illinois Natural History Survey, Prairie Research Institute, University of Illinois, 1816 S. Oak St., Champaign, IL 61820; [2]USDA Forest Service, Northeastern Area, 180 Canfield St., Morgantown, WV 26505; [3]USDA Forest Service, Northern Research Station, 51 Mill Pond Rd. Hamden, CT 06514

14:15 **129** **Quantitative PCR-based method for detecting Nosema bombycis in silkworm egg**
Shunfeng Cai, Xiangkang He, Yin Lu, Xingmeng Lu
(Laboratory of Invertebrate Pathology, Zhejiang University, Hangzhou 310058, China)
(Email: Shyam@zju.edu.cn)

14:30 **130** **The effects of two microsporidian pathogens on the convergent lady beetle, *Hippodamia convergens***
Susan Bjørnson
Biology Department, Saint Mary's University, 923 Robie Street, Halifax, NS B3H 3C3 Canada. susan.bjornson@smu.ca

15:30–16:00 **BREAK** Grand Station III-V

Symposium (Nematodes & NEMASYM) — Wednesday, 16:00-18:00. Haselton

Symbiont contributions to nematode fitness

Organizers: Patricia Stock & Heidi Goodrich-Blair

16:00 **131** ***Drosophila* transcriptional response to infection by *Heterorhabditis* nematodes and their mutualistic *Photorhabdus* bacteria**
Ioannis Eleftherianos and Julio Cesar Castillo
Insect Infection and Immunity Lab, Department of Biological Sciences, Institute for Biomedical Sciences, The George Washington University, Washington DC 20052, USA (ioannise@gwu.edu)

16:30 **132** **A systems biology level analysis of human host adaptation of the nematode symbiont *Photorhabdus asymbiotica.***
Jay Mulley[1], Mike Beeton[2], Nina Ockenden[2], Paul Wilkinson[3], Helge Bode[4] and Nick R. Waterfield[1]
[1] University of Reading, UK. [2] University of Bath, UK. [3] University of Bristol, UK. [4] Goethe Universität, Frankfurt, Germany, [5] Warwick University Medical School, UK. (nickrwaterfield@gmail.com).

17:00 **133** **Natural biology of antimicrobials in symbiotic *Xenorhabdus* species**
Kishore Reddy Venkata Thappeta, Kristin Ciezki and Steven Forst Department of Biological Sciences, University of Wisconsin-Milwaukee, WI 53201 (sforst@uwm.edu)

17:30 **134** **Carrying the Right Symbiont: How Nematode Competitive Success is Influenced by Bacterial Interactions.**
Farrah Bashey Department of Biology, Indiana University

(fbasheyv@indiana.edu)

Contributed Papers | Wednesday, 16:00-17:45. Ellwood

Microbial control 2

Moderator: Stefan Jaronski

16:00 **135 STU** **Temperature, dose and coverage effects on fungal biocontrol of malaria vectors**
R. Heinig[1], K. Paaijmans[2], P. A. Hancock[3], M. B. Thomas[1]
[1]The Pennsylvania State University, State College, PA, USA; [2]Barcelona Centre for International Health Research, Barcelona, Spain; [3]University of Warwick, Coventry, UK (rebecca.heinig@psu.edu)

16:15 **136 STU** **Persistence and efficacy of *Beauveria bassiana*, against the house fly, *Musca domestica* L. on typical structural components of poultry houses**
Naworaj Acharya[1], Rebecca Seliga[1], Edwin G. Rajotte[1], Nina E. Jenkins[1], and Matthew B. Thomas [1, 2]
Department of Entomology[1] and Center for Infectious Disease Dynamics[2], Penn State University, University Park, PA 16802, USA. (nza5060@psu.edu)

16:30 **137** **Preventative solutions for whitefly on seasonal poinsettia cuttings**
Michael Brownbridge[1], Rose Buitenhuis[1], Graeme Murphy[2], Taro Saito and Angela Brommit[1]
[1]Vineland Research and Innovation Centre, 4890 Victoria Avenue North, Box 4000, Vineland Station, Ontario L0R 2E0, Canada; [2]OMAF, Vineland Campus, PO Box 7000, Vineland Station, Ontario L0R 2E0, Canada. (michael.brownbridge@vinelandresearch.com).

16:45 **138** **Commercial formulation of *Metarhizium anisopliae*-based biopesticide (Campaign®) reduces damage by *Maruca vitrata* on cowpea and increases grain yield**
Sunday Ekesi[1], Venansio Tumuhaise[1,2], Samira A. Mohamed[1], Paul N. Ndegwa[2], Lucy W. Irungu[2], Nguya K. Maniania[1]
[1] International Centre of Insect Physiology and Ecology (*icipe*), P.O. Box 30772 - 00100, Nairobi, Kenya
[2] University of Nairobi, P. O. Box 30197 - 00100, Nairobi, Kenya. (sekesi@icipe.org).

17:00 **139 STU** **Transcriptomic Analysis of Tripartite Interactions of *Metarhizium*, *Plasmodium*, and *Anopheles gambiae***
Brian R. Lovett, Joel Vega-Rodriguez, Marcelo Jacobs Lorena and Raymond J. St. Leger
Department of Entomology, 4112 Plant Sciences Building, University of Maryland, College Park, MD 20742. (lovettbr@umd.edu)

17:15 **140** **Field trials using the entomopathogenic fungus *Beauveria bassiana* for the control of UK stored products pests**
Belinda Luke[1], Bryony Taylor[1], Dave Moore[1], Maureen Wakefield[2], Olivier Pontin[3], Pierre Grammare[3] and Clare Storm[4] [1]CABI, Bakeham Lane, Egham, Surrey TW20 9TY, UK [2]FERA, Sand Hutton, York, YO41 1LZ, UK [3]Sylvan Bio, Zl de Tivoli, Route de Mauvières, 37600 Loches, France [4]Exosect, Leylands Business Park, Colden Common, Winchester, Hampshire, SO21 1TH, UK (B.Luke@cabi.org)

17:30 **141 STU** **Field performance of *Brevibacillus laterosporus* and a commercially available *Beauveria* product against brassica pests**
M. Marsha Ormskirk[1], John G. Hampton[1], Phil Rolston[2], Josefina Narciso[1], Stephen Ford[3] and Travis R. Glare[1]
[1] Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand. [2] AgResearch Limited, Agriculture Research Centre, Private Bag 4749, Christchurch 8140, New Zealand. [3] Biotelliga, Unit# 4, 4 Austen Place, Pukekohe, New Zealand. (marsha.ormskirk@lincolnuni.ac.nz)

Contributed Papers | Wednesday, 16:00-17:30. Haselton

Viruses 3

Moderators: Martin A. Erlandson & Madoka Nakai

16:00 **142** **Characterization of a Colombian granulovirus isolated from *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae**
Paola Cuartas[1], Gloria Barrera[2], Laura Villamizar[3]
[1]Biotechnology Ph.D. Student Universidad Nacional de Colombia. Researcher in Biological Control Laboratory. Biotechnology and Bioindustry Center. Colombian Corporation for Agricultural Research. CORPOICA. Mosquera. Colombia. [2]cPh.D. Researcher in Molecular Microbiology Laboratory. Biotechnology and Bioindustry Center. Colombian Corporation for Agricultural Research. CORPOICA. Mosquera. Colombia. [3]Ph. D. Researcher in Biological Control Laboratory. Biotechnology and Bioindustry Center. Colombian Corporation for Agricultural Research. CORPOICA. Mosquera. Colombia. (pcuartas@corpoica.org.co)

16:15 **143** ***Mamestra configurata* nucleopolyhedrovirus-B: Characterization of geographic isolates at the genome sequence level.**
Martin A. Erlandson[1], Matt Links[1], and David A.Theilmann[2]
[1]Saskatoon Research Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2 [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Box 5000, Summerland BC, Canada V0H 1Z0 (martin.erlandson@agr.gc.ca)

16:30 **145** **Detection of native baculovirus *Sf*MNPV in Sinaloa, and their virulence on fall armyworm.**
Cipriano García G1, César Escobedo B1, and Miguel Á. López1.
1Instituto Politécnico Nacional. CIIDIR-IPN Unidad Sinaloa, COFAA. Blvd. Juan de Dios Bátiz Paredes No. 250. Guasave Sinaloa, México. CP.81101 (cgarciag@ipn.mx).

16:45 **146** **Stability and life history parameters of a nucleopolyhedrovirus-resistant strain of the smaller tea tortrix, *Adoxophyes honmai***
Hiroto Shinomiya, Minori Sekiguchi, Yasuhisa Kunimi, Madoka Nakai
Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan. (madoka@cc.tuat.ac.jp)

17:00 **147** **Protein–protein interactions and identification of the A-spike in *Dendrolimus punctatus* cypovirus**
Chuangang Cheng, Yunpeng Shao, Lan Su, Yin Zhou, Xiulian Sun
Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China （sunxl@wh.iov.cn）

17:15 **148** **Molecular characterization of a novel Cypovirus isolated from *Dendrolimus punctatus***
Yin Zhou, Tongcheng Qin, Yuzhou Xiao, Xiulian Sun
Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China （sunxl@wh.iov.cn）

Contributed Papers | Wednesday, 16:00-17:45. Fountainview

Bacteria 3

Moderators: Juan Luis Jurat-Fuentes & Dennis Bideshi

16:00 **149 Toxin production by *Brevibacillus laterosporus,* a potential biocontrol agent of diamondback moth and other insects**
Travis R. Glare[1], M. Marsha Ormskirk[1], John G. Hampton[1] and Murray P Cox[1,2], [1]Bio-Protection Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand. [2]Institute of Molecular Biosciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand. (travis.glare@lincoln.ac.nz)

16:15 **150 Interactions between mosquitocidal Cry4Aa and the brush border membrane proteins of *Culex pipiens* larvae.**
Mohammad T. H. Howlader[1,2], Saori Nakao[2], Hiroshi Sakai[2] and Tohru Hayakawa[2] [1]Department of Entomology, Bangladesh Agricultural University, Mymensisngh-2202, Bangladesh. [2]Laboratory of Gene Engineering, Department of Bioscience and Biotechnology, Graduate School of Natural Science and Technology, Okayama University, Japan. Phone: +81862518205, Fax: +8186251 8264. (tofazzalh@bau.edu.bd)

16:30 **151 Evidence for lateral transfer of cereulide gene cluster by identification of a composite transposon in emetic *B. weihenstephanensis***
Xiaofen Mei[1], Lingling Yang[1], Jacques Mahillon[2], Zhiming Yuan[1], Xiaomin Hu[1]
[1]Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China. Email: huxm@wh.iov.cn
[2]Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, Louvain-la-Neuve, Belgium

16:45 **152 Treat worm infections with crystal protein expressing in probiotic like bacteria**
Yan Hu, Melanie M. Miller, Alan Derman, Brian Ellis, Daniel Huerta, Joseph Pogliano, and Raffi V. Aroian
Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093-0322, U.S.A.(yahu@ucsd.edu)

17:00 **153 Strategies to address corn rootworm control challenges**
William Moar, Matthew Carroll, Graham Head, Tom Clark, and Gerrit Segers
Monsanto Co., 800 North Lindbergh Blvd, St. Louis, MO 63167. (william.moar@monsanto.com)

17:15 **154 Characterization of midgut Cadherin in Bt Cry3A-susceptible and resistant populations of the beetle *Chrysomela tremulae***
David Pauron[1], Manuella Van Munster[1], Sylvie Augustin[2], Yannick Pauchet[3], Marcel Amichot[1], Marie-Paule Esposito[1]
[1]INRA, Centre de recherche PACA, 400 route des Chappes, BP 167, 06903 Sophia Antipolis Cedex, France. [2]INRA, UR 633 Zoologie Forestière, Orléans, France. [3]Max Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, D-07745 Jena, Germany. (david.pauron@sophia.inra.fr)

17:30 **155 STU Inheritance of Cry1F resistance, cross-resistance and frequency of resistant alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae)**
[1]Ana Maria Velez, [1]Terence A. Spencer, [2]Analiza P. Alves, [3]Dan Moellenbeck, [4]Haridas Chirakkal and [1]Blair D. Siegfried
[1] Department of Entomology, University of Nebraska, Lincoln, NE; [2] Dupont Pioneer, Johnston, IA; [3] DM Crop Research group Inc., Polk City, IA; [4] Department of Biological Sciences, University of Nebraska, Lincoln, NE. (anamaria.velez@gmail.com)

SIP Division Business Meetings: 20:00 – 21:30

Viruses & Workshop: Invertebrate virus discovery Grand Station I-II

Bacteria Ellwood

Nematodes Haselton

Fungi Fountainview

Workshop — Wednesday, 20:00-21:30. Grand Station I-II

Viruses

21:00 **156 Deep sequencing technology for arthropod virus discovery**
Sijun Liu[1], Diveena Vijayendran[1], Bryony C Bonning[1]
[1]Department of Entomology, Iowa State University, Ames IA 50011, USA (sliu@iastate.edu)

THURSDAY - 15 August

08:00–17:00 Registration **Lobby**

07:00–08:00 **BREAKFAST** Grand Station III-V

Symposium (Bacteria) Thursday, 08:00-10:00.
Grand Station I-II

Reflections on Bt mode of action

Organizers: Neil Crickmore & Raffi Aroian

08:00 **157 Post-binding events in the mechanism of action of Bt toxins and parallels with mammalian pore-forming toxins**
Raffi V. Aroian and Anand Sitaram, Section of Cell and Developmental Biology, University of California San Diego, USA (raroian@ucsd.edu)

08:30 **158 Role and mechanism of pore formation by *Bacillus thuringiensis* insecticidal crystal toxins**
Vincent Vachon, Raynald Laprade and Jean-Louis Schwartz
Groupe d'étude des protéines membranaires, Université de Montréal, Montreal, and Centre SÈVE, Université de Sherbrooke, Sherbrooke, Quebec, Canada (vincent.vachon@umontreal.ca)

09:00 **159 Learning the ABCs of Bt**
David G. Heckel
Department of Entomology, Max Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, D-07745 Jena, Germany. (heckel@ice.mpg.de)

09:30 **160 Structure/function studies reveal the evolution of pore-forming toxins in bacteria and mammals**
Rodney Tweten
The University of Oklahoma Health Sciences Center, Microbiology & Immunology, Biomedical Research Center, 975 NE 10th St, Oklahoma City, OK 73104 USA

Contributed Papers Thursday, 08:00-10:00.
Ellwood

Viruses 4

Moderators: Robert L. Harrison and Nor Chejanovsky

08:00 **161 Midgut transcriptomic response of the gypsy moth, *Lymantria dispar*, to infection with *L. dispar* and *Autographa californica* multiple nucleopolyhedroviruses**
Robert L. Harrison, Dawn E. Gundersen-Rindal, and Michael E. Sparks
Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, USDA Agricultural Research Service, 10300 Baltimore Avenue, Beltsville, Maryland 20705, USA (Robert.L.Harrison@ars.usda.gov)

08:15 **162 STU First functional annotation of a polydnavirus: the genome of CcBV explored.**
Germain Chevignon[1]; Sébastien Cambier [1]; Julien Thézé [1]; Sébastien Moreau [1]; Elisabeth Huguet [1]; Jean-Michel Drezen [1].
[1]Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Avenue Monge - Parc Grandmont 37200 Tours France. (g.chevignon@gmail.com)

08:30 **163 STU *Spodoptera exigua* MNPV ORF28 blocks viral replication in cell culture.**
Amaya Serrano[1,2], Sarah Nadif[2], Monique van Oers[2], Primitivo Caballero[1], Just M. Vlak[2], and Gorben Pijlman[2]
[1] Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, 31192 Mutilva Baja, Navarra, Spain
[2] Laboratory of Virology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands (amaya.serrano@unavarra.es)

08:45 **164 STU An ODV-specific baculovirus core gene *ha72* is essential for BV production and ODV occlusion**
Huachao Huang[1], Manli Wang[1], Fei Deng[1], Basil M. Arif[2], Hualin Wang[1], Zhihong Hu[1]*
State Key Laboratory of Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, PR China[1]; Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada[2] (huanghuachao99@163.com)

09:00 **165 STU Mapping of the baculovirus AcMNPV ME53 residues essential for its nuclear translocation**
Yang Liu, Eva Nagy and Peter Krell
Department of Molecular and Cellular Biology and Department of Pathobiology, University of Guelph, Guelph ON N1G 2W1

09:15 **166 STU Temporal transcriptional analysis of *Cydia pomonella* granulovirus in the midgut of codling moth by using microarray analysis**
Diana Schneider, Johannes A. Jehle
Institute for Biological Control, Federal Research Centre for Cultivated Plants, Julius Kühn-Institut (JKI), Heinrichstr. 243, 64387 Darmstadt, Germany. E-mail: diana.schneider@jki.bund.de

09:30 **167 STU Genomic adaptation to different hosts - What makes better-adapted viruses?**
Aurélien Chateigner[1]; Cindy Pontleve[1]; Davy Jiolle[1]; Amélie Hébert[1]; Carole Labrousse[1]; Annie Bézier[1]; Elisabeth Herniou[1]
[1]Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 6035, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Avenue Monge - Parc Grandmont 37200 Tours France (aurelien.chateigner@gmail.com)

09:45 **168 STU Characterization of novel sequence in the Infectious Myonecrosis Virus genome *in* Pacific White Shrimp, *Litopenaeus vannamei***
[1,2]Duan Loy, [1]Sijun Liu, [2]Mark Mogler, [3]John Dustin Loy, [1,2]Lyric Bartholomay
Departments of [1]Entomology and [2]Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames IA 50011. [3]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln NE, 68583-0907 (dsloy@iastate.edu)

Contributed Papers Thursday, 08:00-09:00.
Haselton

Fungi 4

Moderators: Ryan Kepler & Steve Rehner

08:00 **169 Deciphering the entomophthoralean genus *Tarichium***
Ann E. Hajek[1], Richard A. Humber[2], and Annette Bruun Jensen[3]
[1]Department of Entomology, Cornell University, Comstock Hall, Ithaca, New York 14853-2601, USA. [2]USDA, ARS, Biological IPM Research, RW Holley Center for Agriculture & Health, Ithaca, New York 14853-2901, USA. [3] Department of Agriculture and Ecology, University of Copenhagen, Frederiksberg C, 1871, Denmark. (aeh4@cornell.edu)

08:15 **170 The *Beauveria bassiana creA* is more than just a carbon catabolite repressor- role in nutrient toxicity, cellular development, and pathogenesis**
Zhibing Luo[1,2], Yuqi Qin[1], Yan Pei[2], and Nemat O. Keyhani[1]
[1]Department of Microbiology and Cell Science, University of

Florida, Gainesville, FL 32611, USA
[2]Key Laboratory of Biotechnology and Crop Quality Improvement of the Ministry of Agriculture of China, Biotechnology Research Center, Southwest University, Chongqing 400716, P.R. China
(luozb1@163.com)

08:30 **171 STU** **Group VIII histidine kinase in *Beauveria bassiana* is essential for the fungal growth, conidiation and adaptation to environment**
Lei Qiu, Sheng-Hua Ying and Ming-Guang Feng
Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, People's Republic of China Address for correspondence: mgfeng@zju.edu.cn

08:45 **173 STU** **Cdc14 phosphatase acts as a nexus of cellular signaling network in *Beauveria bassiana* responses to developmental and stressful cues**
Jie Wang, Sheng-Hua Ying and Ming-Guang Feng
Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, People's Republic of China Address for correspondence: mgfeng@zju.edu.cn

Contributed Papers — Thursday, 08:00-09:30. Fountainview

Nematodes 3

Moderators: Glen Stevens & Racquel Campos-Herreira

08:00 **174 STU** **Evolution of virulence in an entomopathogenic nematode symbiont**
Dana Blackburn[1] and Byron J. Adams[1]
[1]Department of Biology, 401 WIDB, Brigham Young University, Provo, UT 84602, USA. (blackbud@byu.edu)

08:15 **175** **Insect feed effect on entomopathogenic nematode development in the cadaver**
Itamar Glazer[1], Liora Salame[1], Serge-Yan Landau[2], Levana Devash[2], Hassan Azaizeh[3,4], Raghda Mreny[3]; Alex Markovitz[5]
[1]Department of Entomology and Nematology, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel [2]Department of Natural Resources, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel [3]The Institute of Applied Research, The Galilee Society, Shefa Amr 20200, Israel. [4]Tel Hai College, Upper Galilee 12208, Israel.
[5] Kimron Veterinary Institutes, P.O.B. 12, Bet-Dagan, 50250 Israel

08:30 **176** **Desiccation and heat tolerance of entomopathogenic nematodes-Transcriptome analysis**
Yaari Mor[1], Koltai Hinanit[2], Salame Liora[1], Glazer Itamar[1]
[1]Department of Nematology, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel
[2]Department of Ornamental Horticulture, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel

08:45 **177 STU** **New associations between *Deladenus* nematodes, their *Sirex* hosts, and fungal symbionts**
E. Erin Morris[1], Ryan Kepler[1], Stefan Long[1], Elliott Zieman[2], David Williams[3] and Ann E. Hajek[1]
[1]Department of Entomology, Cornell University, Ithaca, New York 14853-2601, USA. [2]Department of Zoology, Southern Illinois University, Carbondale, IL 62901, USA. [3]USDA, APHIS, CPHST, Buzzards Bay, MA 02542-1308, USA. (eem62@cornell.edu)

09:00 **178** **Feltiae/kraussei group of entomopathogenic nematodes: high intraspecific molecular diversity, or a presence of cryptic species?**
Vladimír Půža, Jiří Nermuť, Martina Žurovcová, Lucie Faktorová, Daniela Chundelová, Zdeněk Mráček
Biology Centre ASCR v. v. i., Institute of Entomology, Branišovská 31, 370 05 České Budějovice, Czech Republic, (vpuza@seznam.cz)

09:15 **179 STU** **Can fire gel improve entomopathogenic nematode application?**
Danica F. Maxwell[1], David I. Shapiro-Ilan[1], Edwin E. Lewis[1]
[1]USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008 [1]Dept. Nematology & Dept. of Entomology, University of California - Davis, Davis, CA 95616 (dfmaxwell@ucdavis.edu)

10:00–10:30 **BREAK** Grand Station III-V

Thursday, 10:30-12:30. Grand Station I-II

SOCIETY for INVERTEBRATE PATHOLOGY
Annual Business Meeting & Highlights

12:30–14:00 **LUNCH** On your own

Symposium (Fungi & Bacteria) — Thursday, 14:00-16:00. Grand Station I-II

Ecology of entomopathogenic co-infections

Organizer/Moderator: Christina Nielsen-LeRoux & Helen Hesketh

14:00 **180** **Exploiting entomopathogen co-infections for biological control: current status and future directions**
Helen Hesketh[1], Judith K. Pell[2,3] and Rosemary S. Hails[1]
[1]Centre for Ecology & Hydrology, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, United Kingdom; [2]Agroecology Department, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom;
[3]Current address J.K. Pell Consulting, Luton, Bedfordshire, LU2 7JT, United Kingdom.

14:30 **181** **Interactions between fungi in *Plutella xylostella* larvae - which parameters have the greatest influence on the outcome of dual inoculations?**
Ariel W. Guzman-Franco[1] and Judith K. Pell[2]
[1]Postgrado en Fitosanidad-Entomología y Acarología, Km 36.5 Carretera México-Texcoco, Montecillo, Municipio de Texcoco, Estado de México, 56230, Mexico. [2]J.K. Pell Consulting, Luton, Bedfordshire LU2 7JT, UK, (gariel@colpos.mx)

15:00 **182** **Bacterial-fungal interactions in *C. elegans***
Eleftherios Mylonakis, M.D., Ph.D., FIDSA, Infectious Diseases Division, Alpert Medical School and Brown University, Providence, RI 02903 (emylonakis@lifespan.org).

15:30 **183** **The evolution of virulence with mixed intra- and inter-specific infections in honey bees**
Svjetlana Vojvodic[1,2], Jørgen Eilenberg[2], Annette B. Jensen[2]
[1] Center for Insect Science, University of Arizona, 1041 E. Lowell Street, Tucson, AZ 85721-0106, USA.
[2] Center for Social Evolution, Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frederiksberg C, Denmark.(Vojvodic.sv@gmail.com)

Contributed Papers — Thursday, 14:00-15:15. Ellwood

Microbial Control 3

Moderator: Trevor Jackson

14:00 **184** **Dip treatment using *Isaria fumosorosea*: a potential biopesticide for mitigating the spread of invasive insects on ornamental plants pre- and post-shipping**
Pasco B. Avery[1], Vivek Kumar[2], Luis F. Aristizábal[2], Jean H. Caldwell[1], Cindy L. McKenzie[3], Charles A. Powell[1], Ronald D. Cave[1] and Lance S. Osborne[2]
[1]University of Florida, IFAS, Indian River Research and Education Center, 2199 South Rock Road, Fort Pierce, FL 34945, USA. [2]University of Florida, IFAS, Mid-Florida Research and Education Center, Department of Entomology and Nematology, 2725 Binion Road, Apopka, FL 32703, USA. [3]USDA, ARS, U. S. Horticultural Research Laboratory, Subtropical Insect Research Unit, 2001 South Rock Road, Ft. Pierce, FL 34945, USA. (pbavery@ufl.edu)

14:15 **186** **Integrated use of soil-dwelling predators and microbial biocontrol agents: Compatibility and efficacy against soil-dwelling stages of western flower thrips *Frankliniella occidentalis***
Taro Saito[1], Michael Brownbridge[1], Paul Côté[1]
[1]Vineland Research and Innovation Centre, 4890 Victoria Avenue North, Box 4000, Vineland Station, Ontario, Canada, L0R 2E0. (taro.saito@vinelandresearch.com)

14:30 **187** **Progress in the microbial control of strawberry and vegetable pests: Research and extension efforts in California Central Coast**
Surendra Dara
University of California Cooperative Extension, San Luis Obispo, CA 93401 (skdara@ucdavis.edu)

14:45 **188** **Developing the particle size analysis technique to determine the hydrophobicity of fungal conidia and comparisons with two standard hydrophobicity determining methods**
Belinda Luke[1,2], Jane Faull[2] and Roy Bateman[3]
[1] CABI, Bakeham Lane, Egham, Surrey, TW20 9TY, UK.
[2] Birkbeck College, University of London, Mallet Street, London, WC1E 7HX, UK.
[3] IPARC, c/o Mellingray Annex, Otterham Station, Cornwall, PL32 9YP, UK.
B.Luke@cabi.org

15:00 **189** **Microbial products – New Zealand experiences**
Trevor Jackson
AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand
trevor.jackson@agresearch.co.nz

Contributed Papers — Thursday, 14:00-15:15. Fountainview

Viruses 5

Moderator: James M. Slavicek

14:00 **190** ***Amsacta moorei* entomopoxvirus encodes a functional protein kinase**
Hacer Muratoğlu[a], Duygu Bekircan[b], Remziye Nalçacıoğlu[b], Srini Perera[c], Basil M. Arif[c], Zihni Demirbağ[b] [a]Karadeniz Technical University, Faculty of Science, Department of Molecular Biology and Genetics, 61080 Trabzon, Turkey [b]Karadeniz Technical University, Faculty of Science, Department of Biology, 61080 Trabzon, Turkey [c]Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada zihni@ktu.edu.tr

14:15 **191** **Analysis of the role of the *Lymantria dispar* enhancins on degradation of peritrophic membrane proteins from *L. dispar* larvae**
James M. Slavicek[1], Algimantas Valaitis[1, 2], and Kelli Hoover[3]
[1]Northern Research Station, 359 Main Road, Delaware, OH 43015, USA. [2]Retired. [3]Department of Entomology, Penn State University, 501 Agricultural Sciences & Industries Bldg., University Park, PA 16802, USA. (jslavicek@fs.fed.us)

14:30 **192** **The banchine polydnavirus lineage: distinguishing features and evolutionary history**
Catherine Béliveau[1], Abdelmadjid Djoumad[1], Renée Lapointe[2], Don Stoltz[3], Lisa Kuhn[3], Alejandro Cohen[4], Jean-Michel Drezen[5], Anne-Nathalie Volkoff[6] and Michel Cusson[1]
[1]Natural Resources Canada, Laurentian Forestry Centre, 1055 du PEPS, Québec, QC, Canada; [2]Sylvar Technologies Inc., 1350 Regent St., Fredericton, NB, Canada; [3]Department of Microbiology and Immunology, Dalhousie University, Halifax, NS, Canada; [4]Proteomics Core Facility, Dalhousie University, Halifax, NS, Canada; [5]Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 6035, Faculté des Sciences, Parc de Grandmont, 37200 Tours, France; [6]UMR 1333 INRA, Université Montpellier 2, Place Eugène Bataillon, 34 095 Montpellier, France (michel.cusson@nrcan.gc.ca)

14:45 **193** **Biocontrol of the box tree moth *Cydalima perspectalis*, an invasive pest in Europe, with *Anagrapha falcifera* nucleopolyhedrovirus (AnfaNPV)**
Jana Rose[1], Regina G. Kleespies[1], Yongjie Wang[2§], Jörg T. Wennmann[1], Johannes A. Jehle[1,2,*]
[1]Institute for Biological Control, Federal Research Center for Cultivated Plants, Julius Kühn- Institut (JKI), Heinrichstr. 243, 64287 Darmstadt, Germany (regina.kleespies@jki.bund.de)
[2]Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate (DLR Rheinpfalz), Breitenweg 71, 67435 Neustadt a. d. Weinstrasse, Germany

15:00 **194** **Accumulation kinetics of eight-thymidine mononucleotide repeats of *Anticarsia gemmatalis* multiple nucleopolyhedrovirus *fp25k* during serial passage in Tn5 cells**
Xin-Hua Cheng[1], Dipendra Gautam[1], Tyler Garretson[1], Guo-Hua Huang[1,2], Caitlin Finelli[1] and Xiao-Wen Cheng[1]
[1]Department of Microbiology, 32 Pearson Hall, Miami University, Oxford, Ohio 45056, USA
[2]Institute of Entomology, College of Bio-safety Science and Technology, Hunan Agricultural University, Changsha, Hunan 410128, China (Chengx@MiamiOH.edu)

16:00–16:30 **BREAK** Grand Station III-V

Thursday, 16:30-17:00. Ellwood

Student Business Meeting

18:00–01:00 **BANQUET** Grand Station

FRIDAY - 16 August

07:30–09:00 **BREAKFAST** Grand Station III-V

Friday, 09:00-10:45. Ellwood

NEMASYM workshop

09:00 **195** **Myxococcal multicellular development as a defense against nematode predation**
John L. Dahl, Ph.D., Associate Professor of Biology, University of Minnesota Duluth
[3]USDA, ARS, U. S. Horticultural Research Laboratory, Subtropical Insect Research Unit, 2001 South Rock Road, Ft. Pierce, FL 34945, USA. (pbavery@ufl.edu)

Friday, 09:30-10:45. Ellwood

Contributed papers

See NEMASYM book for abstracts

Moderators: Patricia Stock & Selçuk Hazir

10:45–11:15 **BREAK** Grand Station III-V

Friday, 11:15-12:00. Ellwood

NEMASYM Business Meeting

ORAL ABSTRACTS 2013

IMPORTANT NOTES:

These abstracts should not be considered to be publications and should not be cited in print without the author's permission.

STU indicates papers being judged for graduate student presentation awards

MONDAY - 12 August

PLENARY SYMPOSIUM
Monday, 10:30–12:30

Novel Perspectives on the ecology and evolution of host-pathogen interactions

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

Complexity in the Function and Evolution of Insect Immunity
Brian P. Lazzaro
Dept. Entomology, Cornell University (bplazzaro@cornell.edu)

Individual variation in resistance to infection is ubiquitous in human, plant and animal populations. This variation can have both genetic and non-genetic origins, or, critically, can stem from an interaction between genetic and environmental factors. Using genetic and environmental manipulations, we demonstrate that both dietary nutrition and reproductive status affect defense quality in Drosophila, even though neither of these is expected to act through the canonical immune system. Pleiotropy between seemingly unrelated components if physiology is important because such linkages constrain the evolution of all traits involved. Natural *D. melanogaster* populations harbor genetic variation for immunological sensitivity to reproductive status and dietary perturbation, indicating polymorphism in the genes linking nutritional assimilation and reproduction to defense against infection. Establishing the genetic basis for these linkages and the mechanisms underlying genotype-specific responses to environment is critical to understanding the physiological and evolutionary entirety of defense.

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

Variation in heterogeneity of transmission helps maintain diversity in an insect viral pathogen
Arietta Fleming-Davies[1], Vanja Dukic[2], Brian Rehill [3] and Greg Dwyer[1]
[1] Ecology & Evolution, University of Chicago, 900 E 57th St, Chicago IL 60637, USA. [2] Dept. of Applied Mathematics, 526 UCB, University of Colorado,Boulder, CO 80309, USA. [3] Chemistry Dept., U.S. Naval Academy, 572M Holloway Road, Annapolis, MD 21402, USA. (arietta@uchicago.edu)

Most nucleopolyhedroviruses must kill in order for transmission to occur, and thus they would be expected to evolve to kill as quickly as possible in order to be transmitted sooner. However, the theory of the evolution of virulence predicts that tradeoffs will prevent the evolution of continually increasing virulence. Existing theory does not allow for the coexistence of different strains, and empirical support for tradeoffs is rare. We used the nucleopolyhedrovirus that infects gypsy moths (*Lymantria dispar*) to understand how virus tradeoffs determine observed levels of virulence and permit coexistence. Measurements of virulence for field-collected virus isolates showed high levels of polymorphism in the virus, and slow-killing strains are surprisingly frequent. To understand these patterns, we carried out field experiments to estimate transmission and environmental persistence for 16 virus strains. Mean transmission rates were negatively correlated with environmental persistence, consistent with a tradeoff between these two traits. However, a strong tradeoff between mean transmission and host heterogeneity of transmission appears to be more important in allowing high levels of polymorphism and in ensuring that virus fitness is highest at intermediate transmission rates. The observed increase in host heterogeneity with increased mean transmission appears to be explained by variation across virus strains in larval size at death as a function of speed of kill. Host heterogeneity in transmission thus appears to play a major role in allowing coexistence of multiple pathogen strains and in maintaining intermediate levels of virulence. Variation in virus competitive ability with environmental conditions might also help to promote coexistence.

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

The role of environmental variability in shaping insect immunity and resistance
Courtney C. Murdock
Dept. Entomology, Penn State University, University Park, PA, USA (ccm15@psu.edu)

Insect, parasites, and their hosts associate in a variable world. Recent ecological research has revealed that environmental factors can strongly affect insect immunity and influence the outcome of host-parasite interactions. To date, however, most studies examining insect immune function consider a very narrow set of laboratory conditions and tend to ignore or control environmental variation, rather than explore it. Using a mosquito-malaria system as a model, we demonstrate that insect immunity and resistance are influenced by both a complex interplay of environmental variables. Our results show that individual elements of immune function, as well as composite measures of resistance/virulence can change dramatically with just small variations in environmental conditions. These findings have important implications for interpreting patterns of disease transmission and the potential efficacy of prospective mosquito control tools such as fungal pathogens and *Wolbachia*. More generally, they highlight the need to broaden experimental approaches to better understand the influence of ecological variation in host-parasite interactions.

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

Friendly competition: what happens to the "dilution effect" when hosts compete?
Spencer Hall[1], Alex Strauss[1], Meghan Duffy[2], and Carla Cáceres[3]
[1]Department of Biology, Indiana University, Bloomington IN 47405 USA, sprhall@indiana.edu and atstraus@indiana.edu
[2]Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109 USA, duffymeg@umich.edu
[3]Department of Animal Biology, University of Illinois, Urbana, IL 61801 USA, caceres@life.illinois.edu

Other species can dampen disease outbreaks in host populations through several different mechanisms. For instance, consider the "dilution effect" in systems in which hosts consume parasites with free-living infectious stages (spores). In this mechanism, resistant, "diluter" hosts can reduce disease prevalence in focal hosts by removing but not producing spores. But what happens when both host types compete for shared resources? We consider this "friendly competition" scenario in a zooplankton host – fungal parasite system. More specifically, three clonal genotypes of the focal host-grazer (*Daphnia dentifera*), themselves varying in infection risk, experienced friendly competition with a more resistant grazer (*Ceriodaphnia* sp.) during epidemics of an obligate killer (*Metschnikowia bicuspidata*). Both dynamical models and mesocosms showed three suites of results. First, the success of the dilution effect (i.e., lower prevalence in systems with the friendly competitor) depended on susceptibility of the focal host genotype. When the focal host was highly susceptible, prevalence remained high with the friendly competitor. Thus, the dilution effect failed. Conversely, prevalence was low with more resistant focal hosts, so dilution was not effective then, either. Dilution did succeed with the focal host having intermediate resistance. Second, the competition component of friendly competition reduced density of uninfected focal hosts during epidemics no matter what - a negative outcome from a conservation standpoint. Third, small epidemics started in populations of the diluter competing with highly susceptible hosts – a third negative outcome. These outcomes reveal pronounced limitations of the dilution effect for disease control among competitors.

SYMPOSIUM (Cross Divisional)
Monday, 14:00-16:00

Invertebrate Innate Immunity

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

Regulation of hemolymph protease cascades in the immune system of *Manduca sexta*

Michael R. Kanost
Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS 66506 USA (kanost@ksu.edu)

Extracellular serine protease cascades mediate rapid responses to infection and wounding in animals. Such cascade pathways offer mechanisms for rapid, local amplification of a small initial signal, with potential for regulation at multiple levels. The proteases circulate as inactive zymogens and become sequentially activated upon recognition of aberrant tissues or microbial polysaccharides. After accomplishing their functions, the active enzymes are often inactivated by serine protease inhibitors. It is becoming apparent that protease cascade pathways in blood are often connected, creating protease "webs," and this is likely to be true in *Manduca sexta* and other insect species. Activation of serine proteases in hemolymph is a component of several types of lepidopteran immune responses, including melanotic encapsulation, activation of antimicrobial peptide synthesis, and modulation of hemocyte function. Many insect hemolymph serine proteases are composed of a carboxyl-terminal protease domain and an amino-terminal clip domain. Clip domains are 35-55 amino acid residue sequences that may mediate interactions of members of protease cascade pathways. We have cloned cDNAs for more than 20 clip domain proteases expressed in fat body or hemocytes of *Manduca sexta* and are investigating their functions in immune responses and their regulation by members of the serpin family of protease inhibitors. Hemolymph protease pathways leading to activation of prophenoloxidase and activation of the cytokine, spätzle, will be discussed, as well as regulation of these pathways by inhibitory serpins. Initial observations on hemolymph proteases and serpins from the *Manduca sexta* genome sequence will be provided.

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

Defense Responses of *Biomphalaria* to Schistosomes

Eric S. Loker
Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131, USA (esloker@unm.edu)

Thanks to the impetus provided by the pioneering work of Miyairi and Suzuki that was undertaken one hundred years ago, we have since learned a great deal about the basic biology of schistosomes and their interactions with snails. For example, molecular phylogenetic studies have shown the extent to which host switching among snails of various families has played a role in diversification of schistosomes. This macroevolutionary pattern is perplexing though, given the high degree of fidelity to their snail hosts demonstrated by lab studies of extant schistosomes. Understanding the mechanisms involved in governing compatibility between snails and schistosomes provides one way to develop a more comprehensive picture of how schistosomes have evolved, and how they persist in the modern world. We have studied interactions between the trematodes *Echinostoma paraensei* and *Schistosoma mansoni*, and their common snail host *Biomphalaria glabrata*, to reveal the general nature of gastropod immunity and the specific nature of anti-schistosome defenses. Successful infection by either trematode species results in down-regulation of many snail immune features. If, however, the parasites are successfully resisted by the snail, then a pattern of up-regulation of immune features is seen. Among the prominent features involved are fibrinogen-related proteins, or FREPs, molecules with IgSF and fibrinogen domains. Snails are capable of generating diversified FREP molecules that have opsonic and adhesive properties consistent with a role in defense. When expression of one particular FREP, FREP3, is knocked down using RNAi, susceptibility to infection can be markedly enhanced. These studies help envision how schistosomes exhibit specificity in their interactions with snails, yet may have been compromised in the past, enabling relationships with new snails to develop. Dr. Miyairi would be pleased that his seminal work continues to attract considerable interest from the world's parasitologists and immunologists interested in host-parasite interactions. Supported by NIH grant AI101438 and 1P20RR18754.

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

Multiple microbes and immunity in honey bees

Jay D. Evans, Ryan S. Schwarz
USDA-ARS Bee Research Lab BARC-East Bldg 306 Beltsville, MD USA 20705 (jay.evans@ars.usda.gov)

No insects live in a vacuum and honey bees are regularly exposed to multiple parasites and pathogens simultaneously. Honey bees also maintain a stable contingent of intestinal bacteria and other microbes that might mitigate or exacerbate disease risks. There has been recent progress in determining how paired microbes interact directly during the course of infection. As one example, several controlled studies have shown that bacteria symbionts of honey bees can affect the course of larval foulbrood disease through *direct* interactions. Here, we will focus on *indirect* effects of co-exposure to microbes, by describing bee immune responses following exposure to 1) two gut microbes (*Nosema ceranae* and *Crithidia mellificae*), 2) gut bacterial symbionts followed by viral or parasite exposure, and 3) bacterial pathogens during larval development. These studies offer insights into how bees mount (when they can) specific immune responses, and whether certain combinations of biotic threats are especially dangerous. We will place this work in the context of genetic components on bee immunity as well as other work describing the impacts of *Varroa* mites, chemicals, and nutritional stress on bee immunity.

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

Antiviral defense in aphids

Bryony C. Bonning[1], Diveena Vijayendran[1], Sijun Liu[1]
[1]Department of Entomology, Iowa State University, Ames, IA 50011, USA. (bbonning@iastate.edu)

The pea aphid, *Acyrthosiphon pisum*, has become a model hemipteran and genes encoding all of the major components of the RNA interference (RNAi) pathway are present in the genome. Transcriptomic and small RNA analyses revealed the presence of viruses in all four aphid species that we have in laboratory cultures. In most cases, the typical 22 nt peak of virus-derived small RNA (vsRNA) was seen indicating degradation of viral RNA by the RNAi pathway. In contrast, the small RNA profile for the new virus Acyrthosiphon pisum virus-2 (APV-2; Dicistroviridae), was distinct. Evidence for a new antiviral pathway in aphids will be presented.

CONTRIBUTED PAPERS
Monday, 14:00-16:00

Nematodes 1

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

Entomopathogenic nematodes and soil food webs: Natural assemblages and specific niche associations in natural *versus* agricultural areas

Raquel Campos-Herrera[1,2], Fahiem E. El-Borai[1,3], and Larry W. Duncan[1]
[1]Departamento de Contaminación Ambiental, Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Ciencias Agrarias (ICA), 28006, Madrid (Spain), [2] Citrus Research and Education Center, University of Florida, 33850, Lake Alfred (Florida, USA), [3] Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt. (raquel.campos@ica.csic.es; r.camposherrera@ufl.edu)

Native entomopathogenic nematodes (EPNs) are distributed in natural and agricultural ecosystems worldwide. Our objective here was to identify differences in the assemblages and niche associations of EPN food webs in natural (n = 90) and citrus (n = 53) areas surveyed during summer-fall 2009-2011. We used species–specific qPCR probes for 13 EPN species, 2 species of *Paenibacillus* (ectoparasitically associated with EPNs), 7 species of nematophagous fungi (NF), and free–living bacteriophagous nematodes (*Acrobeloides*–group), some of which compete with EPNs. The abundance

of EPNs, NF and *Acrobeloides*-group species and their ecological indices (richness, Shannon-Weiver biodiversity and evenness) were higher in citrus orchards than in natural areas ($P < 0.05$). *Steinernema glaseri* and *H. floridensis* were encountered only in natural areas and *S. riobrave* and *S. scapterisici* only in citrus orchards, whereas 5 species occurred in both types of habitat. *S. diaprepesi* and *H. indica* were especially more prevalent in citrus groves ($P < 0.001$). All NF species occurred at higher levels in nematode samples from citrus orchards ($P < 0.001$) except *Paecillomyces lilacinus* which was more abundant among nematodes recovered from natural areas ($P = 0.02$). *Paenibacillus* sp. was associated with *S. diaprepesi* in citrus and with *Steinernema* sp. in natural areas ($P < 0.05$), suggesting a possible niche adaptation. Some of the differences in species-habitat associations may help support the co-habitation of the Florida peninsula by closely related EPN species. Additional analysis of the soil properties will contribute to understanding the basis of these habitat preferences.

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

Storage temperature and duration affect *Steinernema scarabaei* dispersal and attraction, virulence, and infectivity to a white grub host

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The entomopathogenic nematode *Steinernema scarabaei* has exceptional potential for the control of many white grub species. The objective of this study was to develop a better understanding of the dispersal behavior of *S. scarabaei* IJs and the influence of storage conditions on its dispersal and infectivity. We found that storage temperature and duration had a strong effect on *S. scarabaei* IJ dispersal and virulence and infectivity to third-instar oriental beetle, *Anomala orientalis*. But even under conditions conducive to movement only a small proportion of IJs moved towards a host. IJ dispersal declined with storage time by a factor of around 100 between 1 and 12 weeks of storage whether the IJs were stored at room temperature or 8 °C; however, the decline was more than twice as fast after storage at 8 °C. Host attraction also diminished with storage duration. IJ virulence and infectivity declined with storage time for IJs stored at room temperature. In contrast, for IJs stored at 8 °C virulence remained high and infectivity increased over time. The decrease in dispersal and infectivity when stored at room temperature may reflect an adaptation to conserve energy in the absence of hosts since *S. scarabaei* IJs have to persist through extended periods in summer during which infections are unlikely to occur. The even faster decrease in dispersal rate when stored at 8 °C may suggest a cold-induced dormancy that may serve as an overwintering strategy. The parallel increase in infectivity, however, seems to contradict such a strategy.

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

Parasitism of *Sirex noctilio* by non-sterilizing *Deladenus siricidicola* in northeastern North America

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The parasitic nematode *Deladenus siricidicola* has been extensively used for biological control of the invasive woodwasp *Sirex noctilio* in the Southern Hemisphere. The strain of *D. siricidicola* used for biological control sterilizes *S. noctilio* females, although non-sterilizing strains of this nematode also occur. A non-sterilizing (NS) strain is established in the most recent invasion of *S. noctilio*, in northeastern North America. This study analyzed the effects of the NS strain of *D. siricidicola* on invasive *S. noctilio* collected from New York and Pennsylvania. Nematode parasitism had both a direct negative effect on the number of eggs produced by adult female *S.* noctilio and an indirect negative effect, due to smaller size in nematode-parasitized females. *S. noctilio* with NS nematodes were found in 44.0% of trees and 26.9% of all individuals diagnosed, reaching 27.9 $\pm$ 26.0 % parasitism (mean $\pm$ S.D.) when averaged across sites. There was greater parasitism of female *S. noctilio* than males. We also compared parasitism by hymenopteran parasitoids to NS parasitism. Parasitism by nematodes averaged 31.9 $\pm$ 35.4% per tree, while parasitism by hymenopteran parasitoids averaged 41.8 $\pm$ 19.6%. NS *D. siricidicola* may be a less effective biological control agent than the sterilizing *D. siricidicola* or parasitic hymenopterans.

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

Community composition of entomopathogenic nematodes associated with Vaccinium spp. roots in cultivated and wild settings

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Entomopathogenic nematodes (EPNs) can be viable biocontrol agents of root pests in agricultural crops. Recent studies in maize and citrus have shown the ability of EPNs to utilize herbivore-induced responses from plant roots to locate insect hosts belowground; however, thus far no studies have considered natural habitats. In New Jersey's Pinelands National Reserve (PNR), the natural populations of highbush blueberry (*Vaccinium corymbosum*) and lowbush blueberry (*Vaccinium angustifolium*) from which most popular highbush blueberry varieties were domesticated are commonly found adjacent to commercial blueberry fields. In this agroecosystem growers treat the soil with insecticide to manage the oriental beetle (*Anomala orientalis*), an invasive, prevalent root feeding pest. We investigated the effects of plant domestication on belowground plant induced responses to *A. orientalis* feeding and compared differences in species diversity of EPNs between natural and agricultural blueberry habitats. Soil was sampled and plants were propagated from 5-10 pairs of domesticated and wild blueberry plant populations in the PNR. Soil samples taken from blueberry roots were baited with wax moth (*Galleria mellonella*) larvae to confirm activity and presence of endemic EPNs as well as extracted with sugar floatation for to use in molecular identification using PCR. The overall trend was for a higher EPN prevalence in soil from cultivated fields but a more diverse EPN community in natural stands.

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

Influence of the Natural Microbiome on Nematode Growth in the Wild

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Like us, *C. elegans* lives in a microbial world. In its natural habitats of rotting fruits and vegetation, these nematodes proliferate as they dine on an array of microbes. Interactions with microbes span a spectrum from constant confrontation (pathogens) to relative indifference (food) and perhaps even mutual benefit (symbionts). This study identifies these natural microbes and addresses whether microbiome composition influences proliferation of *C. elegans* in the wild. To examine this question, we sequenced bacterial 16S (SSU) rDNA amplicons from habitats with wild *C. elegans* populations collected in France and Spain. Our results show that *C. elegans* encounters a broad array of bacteria in the wild—especially the divisions (phyla) of Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria. Abundance-weighted comparisons of phylogenetic differences (UniFrac) showed distinct clustering by habitat-type. Interestingly, rotting apples clustered by population state of the worms [proliferating vs. non-proliferating (dauer)]. Proliferating *C. elegans* appear in apples with 'simpler' microbiomes (lower diversity, fewer species and Proteobacteria-rich). Specific alpha-proteobacteria were also enriched in apples with proliferating worms, while a diverse array of genera characterize apples with non-proliferating worms (e.g., *Pseudomonas*, and several Bacteroidetes). Population size also correlated with apple rottenness, indicating bacterial load is important as well. Similarly, Proteobacteria content does affect *C. elegans* growth rate in

the lab, as worms grew faster on mixtures (and isolates) with 80% Proteobacteria versus those with 40% Proteobacteria. Together, these studies define the microbial diet of *C. elegans* and implicate the natural microbiome as a key determinant of *C. elegans'* growth in the wild.

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

Group behavior in insect parasitic nematode dispersal

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Movement behavior is critical to determination of spatial ecology and success of foraging in predators and parasites. In this study movement behavior of entomopathogenic nematodes was explored. Movement patterns in sand were investigated when nematodes were applied to a specific locus or when the nematodes emerged naturally from infected insect host; six nematode species were tested (*Heterorhabditis bacteriophora*, *H. indica*, *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri*, and *S. riobrave*). We discovered that nematode dispersal followed an aggregate pattern rather than a random or uniform distribution. These findings have implications for parasitic nematode spatial distribution and suggest that group behavior is involved in nematode foraging.

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

Entomopathogenic nematode attraction to the chemical cues produced by cadavers.

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Entomopathogenic nematodes (EPN) are exposed to a range of cues in the soil. If these cues are positively associated with the presence of insect hosts, one might hypothesize that EPN would respond positively to such cues. Decomposing animals release many different chemical compounds into the soil, attract large numbers of foraging insects, and produce large numbers of insect larvae. Thus, these chemical compounds may serve as an important cue for foraging EPN. We hypothesized the *Steinernema feltiae* IJs would respond positively to two particular compounds (putrescine and cadaverine) produced during cadaver decomposition. This hypothesis was justified based on the association of *S. feltiae* with flies, the significance of flies and maggots in the decomposer community, and the tendency of these high molecular weight compounds to diffuse downwards into the soil. We used standard agar-based "bulls-eye" attraction assays, and assessed *S. feltiae* responses to diffusion discs soaked in 5 µl of 50 µmol, 100 µmol, and 500 µmol concentrations of each of the two compounds. In general, putrescine appeared to repel IJs (average attraction index score of -0.50), while cadaverine attracted them (average attraction index score of 0.35). The strongest response was at the 100 µmol concentration for both compounds. We hypothesize that the differential IJ response to the two compounds may relate to the specific timing of the compound's production during decomposition, as it relates to the dispersal of maggots from the cadaver. Future investigations are focused on conducting these trials in a three-dimensional medium.

Contributed paper. Monday, 15:30. **16 STU**

Potential Natural Enemies of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae)

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Top-down effects of different soil arthropods on entomopathogenic nematode populations were tested in laboratory conditions in two ways. Experiments showed that crickets (*Gryllus bimaculatus*), American cockroaches (*Periplaneta americana*), ants (*Tetramorium chefketi* and *Pheidole pallidula*), earwigs, mites (*Sancassania polyphyllae*) and springtails (*Sinella curviseta*) have different responses to nematode-killed insects. Results suggested that ants (*Tetramorium chefketi*), cockroaches, mites and earwigs fed on *Steinernema*-killed insects whereas neither crickets nor springtails consumed them. Although crickets and both ant species used in the studies were deterred from 2-day-old *Heterorhabditis*-killed insects, cockroaches consumed 58.3% of the cadavers. In the second part of the study, experiments were conducted to determine whether mites and springtails consume infective juveniles of entomopathogenic nematodes. Results showed that *S.polyphyllae* mites are not specialist predators of infective juveniles in soil, whereas springtail species *Sinella curviseta* and *Folsomia candida* consumed significant numbers of the infective juveniles they encountered in this experiment. The overall results show that top-down regulatory processes can be a limiting factor for EPN populations under laboratory conditions.

CONTRIBUTED PAPERS
Monday, 14:00-16:00

Bacteria 1

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

Comprehensive analysis of gene expression profiles of the beet armyworm *Spodoptera exigua* larvae challenged with *Bacillus thuringiensis* Vip3Aa toxin

Yolanda Bel[1], Agata K. Jakubowska[1], Juliana Costa[2], Salvador Herrero[1] and Baltasar Escriche[1]
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Host-pathogen interactions form complex relationship of which many aspects are not completely understood. Vip are insecticidal *Bacillus thuringensis* (Bt) proteins that represent an interesting alternative to the classical Bt Cry toxins because the data available so far suggest that they do not share the same mode of action. A genome-wide microarray of the beet armyworm *S. exigua* was used to determine host genes that respond to Vip3Aa intoxication, by evaluating the changes in gene expression levels caused by a sublethal doses treatment. Results showed that the toxin induced a wide transcriptome response, as a 19% of unigenes in the microarray responded significantly to the treatment. The number of up and down-regulated unigenes was very similar but the levels of down regulation were higher than the maximum levels of up-regulation found. The up-regulated sequences were enriched in pathogen response genes and genes involved in innate immune response, such as antimicrobial peptides (AMPs) and *repat* genes. The down-regulated sequences were mainly unigenes homologous to proteins involved in metabolism. Genes related to the mode of action of Bt Cry proteins were found generally slightly overexpressed after Vip3Aa treatment. Presented study is the first wide-genome analysis of the response of lepidopteran insects to Vip3Aa toxin intoxication. An insight into the molecular mechanisms and components related to Vip intoxication will allow to design more effective management strategies for pest control.

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

Retargeting of the Bt toxin Cyt2Aa against hemipteran insect pests.

Nanasaheb P. Chougule[1], Huarong Li[1,2], Sijun Liu[1], Lucas B, Linz[1], Kenneth E. Narva[2] , Thomas Meade[2], Bryony C. Bonning[1]
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The sap sucking insects (Hemiptera) are not particularly susceptible to Bt toxins. We demonstrate a novel strategy for modification of the Bt toxin

Cyt2Aa to improve toxicity against two hemipteran pests, the pea aphid (*Acyrthosiphon pisum*) and the green pea aphid (*Myzus persciae*). A pea aphid gut binding peptide (GBP3.1), which binds to membrane alanyl aminopeptidase-N (APN) on the midgut and hindgut epithelia, was incorporated into Cyt2Aa to enhance binding and increase toxicity against aphids. Two sets of modified Cyt2Aa were constructed, by addition of GBP3.1 to six surface exposed loops in Cyt2Aa or by substitution of amino acids in each Cyt2Aa loop with GBP3.1. Only five of the eleven modified toxins retained toxicity against *Aedes aegypti* larvae at levels comparable to that of Cyt2Aa. All modified toxins demonstrated enhanced binding to pea aphid gut brush border membrane vesicles (BBMV) in pull down and surface plasmon resonance assays. The five toxins that retained toxicity against *Ae. aegypti* showed increased toxicity against *A. pisum* and three showed increased toxicity against *M. persciae* in membrane feeding assays. The modified toxins caused extensive damage to the midgut epithelium. This strategy may allow for transgenic plant-mediated suppression of other agriculturally important hemipteran pests.

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

Differential binding of Cry1Ab and Cry1Fa proteins from *Bacillus thuringiensis* to five aminopeptidases N from *Ostrinia nubilalis* (Hübner)

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The European corn borer (*Ostrinia nubilalis*) is an important pest of cultivated maize. Cry1Ab and Cry1Fa proteins from *Bacillus thuringiensis* are highly toxic against this insect species and this has led to the development of transgenic insect-resistant plants (Bt-corn) expressing these proteins. A key step in the mode of action of Cry proteins is their interaction with larval midgut membrane receptors. In the present work, the receptors for Cry1Ab and Cry1Fa in *O.nubilalis* have been studied. Ligand blot analysis of brush border membrane proteins showed that both Cry proteins mainly bound to a 150 kDa-band whereas Cry1Fa also bound to a 75 kDa-band. LC-MS/MS analysis of the SDS-PAGE excised bands identified several aminopeptidase N (APN) isoforms. Previous research proposed APNs as receptors for Cry proteins in several insect species. These enzymes belong to a protein family with at least eight different members that are expressed simultaneously in the midgut of lepidopteran larvae. We have previously described the expression of five *O. nubilalis* APNs in *Sf*21 insect cells using a baculovirus system. Now, the interaction of such individually expressed proteins with Cry1Ab and Cry1Fa was evaluated by immunocytochemistry. Binding analysis showed that *On*APN1 interacted with both Cry1Ab and Cry1Fa, whereas *On*APN3a and *On*APN8 only bound to Cry1Fa. Two isoforms, *On*APN2 and *On*APN3b, did not interact with any of these two proteins. This work provides the first direct evidence of a differential role of *On*APN isoforms in the mode of action of Cry proteins.

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

Genome characterizing of mosquitocidal *Bacillus thuringensis* isolate S2160-1

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Bacillus thuringiensis mosquitocidal isolate S2160-1 isolated from in earth of subtropical nature reserve in Guangxi. Previous studies reported that the isolate S2160-1 has much higher mosquitocidal toxicity to Anopheles (*Aedes albopictus*) than that of *Bacillus thuringiensis israelensis* (Bti) and four cry genes, *cry30Ea, Cry30Ga, cry50Ba and cry54Ba* were identified as well. The plasmid profile of S2160-1 was complete different with Bti. *Bacillus thuringiensis* S2160-1 isolate was proposed to be an alternative to Bti. The genome of Bt S2160-1 was sequenced by the Illumina HiSeq2000 sequencer to discover all of insecticidal genes as well as to explain high mosquitocidal activity at genomic level. In this study, we obtained a 703Mb high quality reads (112 folds genomic coverage) to be assembled 82 Scaffolds (N50=267kb), and 207 contigs (N50=105kb), among them, 81 Scaffolds and 206 contigs with length of >500bp. The whole genome size is about 6.3M (GC%=34.81). Preliminary analysis showed that the chromosome size of S2160-1 isolates is about 5.5Mb, containing predicted 5629 ORFs; while the plasmid genome size is about 850kb, containing predicted 1042 ORFs, Blast analysis showed that S2160-1 isolates may have a toxic protein pathogenicity island, harboring at least 8 genes encoding toxic crystal proteins including Cry 4C and Cry 10A. Based on the phylogenetic analysis, the chromosome of the sequenced isolate seems to be the closest to the Bt strains of Bt 407, CT43 and IS5056 within *Bacillus thuringiensis* and much more closely related to *Bacillus anthracis* Ames than that of *Bacillus cereus* ATCC14579.

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

A novel microcapsule formulation: alkaline releasing for *Bacillus thuringiensis* insecticidal proteins

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Crystal proteins synthesized by *Bacillus thuringiensis* (Bt) have been used as biopesticides because of their toxicity to the insect larval hosts. To protect the proteins from environmental stress to extend their activity, we have developed a new microcapsule formulation. Poly (acrylic acid) (PAH) and poly (styrene sulfonate) (PSS) were fabricated through layer-by-layer self-assembly based on a $CaCO_3$ core. Cry1Ac protoxins were loaded into microcapsules through layer-by-layer self-assembly at low pH, and the encapsulated product was stored in water at 4°C. Scanning electron microscopy (SEM) was used to observe the morphology of the capsules. To confirm the successful encapsulation, the loading results were observed with a confocal laser scattering microscope (CLSM), using fluorescein-labeled Cry1Ac protoxin (FITC-Cry1Ac). The protoxins were released from the capsule under the alkaline condition corresponding to the midgut of certain insects, a condition which seldom exists elsewhere in the environment. The following bioassay experiment demonstrated that the microcapsules with Cry1Ac protoxins displayed approximately equivalent insecticidal activity to the Asian corn borer compared with free Cry1Ac protoxins, and empty capsules proved to have no effect on insects. Further result also indicated that the formulation could keep stable under the condition of heat and desiccation. These results suggest that this formulation provides a promising methodology that protects protoxins from the environment and releases them specifically in the target insects' midgut, which has shown potential as biopesticide in the field

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

Importance of alkaline phosphatase in the mode of action of Cry1 toxins

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The mode of action of Cry1 insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) includes a necessary step of binding to proteins on the surface of the insect midgut cells. This binding step is conducive to enterocyte death, which disrupts the integrity of the epithelial barrier and

allows the onset of septicemia and ultimately insect death. Data supporting a functional Cry1 receptor role have been reported for diverse proteins, including cadherin, aminopeptidase-N (APN), and ATP binding cassette transporter isoform 2 (ABCC2). In this presentation we will report data supporting the functional role of ALP in diverse lepidopteran species and present evidence of the importance of this protein in the Cry1 mode of action.

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

Cross-order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins

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The increasing number of *B. thuringiensis* proteins with pesticidal activities across orders and phyla raises the question how widespread cross-activities are and if they are of sufficient biological significance to have implications for ecological safety of those proteins in pest control applications. Cross-activity is reported for 27 proteins and 68 taxa but is substantiated by reasonable evidence (mortality estimates) in only 19 cases involving 44 taxa. Cross-activities occur in 13 primary rank families across all three classes of pesticidal proteins (Cry, Cyt and Vip), and comprise 14 proteins affecting species across two orders, four proteins affecting three orders and one protein affecting four orders, all within the class Insecta. Cross-activity was quantified (LC_{50} estimates) for 16 proteins and 25 taxa. Compared to toxicity ranges established for Diptera-, Coleoptera-, Lepidoptera- and Nematoda-active proteins, 11 cross-activities are in the low-toxicity range (10 – 1000 µg/ml), 12 in the medium- (0.10 – 10 µg/ml) and two in the high-toxicity range (0.01 – 0.10 µg/ml). Although cross-activities need to be viewed with caution until they are confirmed through independent testing, current evidence suggests that cross-activity of *B. thuringiensis* pesticidal proteins needs to be taken into consideration when designing and approving their use in pest control applications.

Contributed paper. Monday, 15:45. **24 STU**

The insecticidal specificity of Cry1Ah protein

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cry1Ah1, a novel holo-type gene cloned by our lab, encoded a protein exhibited strong toxicity against many lepidopteran insect except *Bombyx mori*, was already applied in transgenic rice and maize. The identity between Cry1Ah and Cry1Ai is 84%. They share a very similar structure but exhibit "contrary" function. We analyze the binding protein on BBMVs using an improved receptor-fishing method and constructed Loop-interchange mutants of Cry1Ah and Cry1Ai to explore the mechanism of insecticidal specificity. The results showed there was a special binding band-APN1 of Cry1Ah on BBMVs from *H. armigera* compared with *B. mori*. The bioinformatics analysis of APN1 shows there are 36 O-linked glycosylation sites on APN1 cloned from *H. armigera*, while only five predicted sites on APN1 from *B. mori*. The carbohydrate structure may cause Cry1Ah binding to APN1 from *H. armigera* with high affinity. According to the compare of the predicted structure and amino acid sequences between Cry1Ah and Cry1Ai, we constructed some Loop mutants. The bioassay of Loop mutants against *H. armigera* showed that changing of Loop 2 contributed to the alternative toxicity (Cry1Aihloop2: LC_{50}=64.23 µg/g; Cry1Ahiloop2: LC_{50}>500 µg/g), while changing of Loop 3 caused lost of toxicity (Cry1Aihloop3: LC_{50}>500 µg/g; Cry1Ahiloop3: LC_{50}>500 µg/g). It suggested that Loop 3 is very important to the toxicity and Loop 2 is related to insecticidal specificity. To sum up, the insecticidal specificity of Cry1Ah may be due to the different amino acid on Loop 2, and binding to APN1with different affinity.

CONTRIBUTED PAPERS
Monday, 14:00-15:45

Fungi 1

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

Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect pathogenic fungi

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Symbiotic nitrogen transfer in soil has largely focused on nitrogen fixing bacteria. Vascular plants can lose a substantial amount of this nitrogen through insect herbivory that is then incorporated into the insect. Certain plants were shown to be able to re-acquire this nitrogen through a partnership with the endophytic, insect pathogenic fungus (EIPF) *Metarhizium robertsii*. That is, the endophytic capability and insect pathogenicity of *M. robertsii* are coupled to provide an active method of nitrogen transfer to plant hosts via fungal mycelia. We analyzed the ubiquity of this nitrogen transfer in five *Metarhizium* species representing those with broad (*M. robertsii, M. brunneum and M. guizhouense*) and narrower insect host ranges (*M. acridum* and *M. flavoviride*) as well as *Beauveria bassiana* and *Verticillium lecanii.* Insects were injected with ^{15}N-labeled nitrogen, and we tracked the incorporation of ^{15}N into dicots, haricot bean (*Phaseolus vulgaris*) soybean (*Glycine max)* and monocots, switchgrass (*Panicum virgatum*) and wheat (*Triticum aestivum),* in the presence of these fungi in soil microcosms. All *Metarhizium* species and *Beauveria bassiana* showed the capacity to transfer nitrogen to plants, although to varying degrees. Endophytic association by these fungi increased overall plant productivity. We also show that *M. robertsii* is able to transfer insect derived nitrogen to plants in the field, where microbial competition is potentially high. *Metarhizium spp*. and *Beauveria bassiana* have a worldwide distribution, ranging from arctic to tropical habitats, with high soil abundance, and may play an important role in the ecological cycling of insect nitrogen back to plant communities.

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

Evolutionary forces acting on endophytic insect pathogenic fungi

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Metarhizium and *Beauveria* are well known insect pathogens and evidence also strongly supports these fungi as endophytes. Thus these fungi have, at least, bifunctional lifestyles. But which lifestyle, as an insect pathogen or endophyte, has a greater role in the evolution and divergence of these fungi? *Metarhizium* is phylogenetically related to the fungal grass endosymbiont, *Epichloë* and suggests endophytism as an evolutionary diverging point. Since the time of divergence from *Epichloë* (ca. 100 million years ago) *Metarhizium* and *Beauveria* have also evolved as insect pathogens. We have shown that the endophytic capability and insect pathogenicity of these fungi are coupled to provide an active method of insect-derived nitrogen transfer to plant hosts. That is, there is a symbiotic relationship between fungus and plant. Here we explore the potential evolutionary dynamics of plant benefits of this association, fungal benefits of the association, and evolutionary forces potentially driving insect host ranges in the fungal-plant relationship. While other abiotic and biotic factors cannot be excluded in contributing to species divergences, it appears that plant relationships have been a driving factor in the evolution of *Metarhizium* species and *Beauveria*.

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

Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae)

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Ten fungal isolates belonging to the genera *Beauveria* , *Hypocrea*, *Gibberella*, *Metarhizium*, *Trichoderma* and *Fusarium* were evaluated in the laboratory for their ability to endophytically colonize *Vicia faba* and *Phaseolus vulgaris* and to assess their possible negative effects on leafminers. *Beauveria* (ICIPE279), *Hypocrea*, *Gibberella*, *Fusarium* and *Trichoderma* isolates colonized roots, stems, and leaves of both host plants. *Beauveria* isolates G1LU3 and S4SU1 colonized roots, stems, and leaves of *P. vulgaris* but only the root and stem of *V. faba*. Isolates of *Metarhizium* failed to colonize the two host plants. The effects of endophytically-colonized fungal pathogens on mortality, oviposition, emergence and longevity of *L. huidobrensis* were investigated after endophytic colonization of *V. faba* plants. All the fungal isolates that succeeded in colonizing the host plant were pathogenic to *L. huidobrensis*, causing 100% mortality within 13.2 ± 0.7 - 15.0 ± 0.6 days. However, *Hypocrea* outperformed the other isolates ($P < 0.0001$) in reducing longevity of the progeny (11.2 ± 1.0 vs. 17.8 ± 1.4 days in the control), the number of pupae (80.0 ± 6.7 vs. 387.0 ± 21.7 pupae in the control), and adult longevity (3.8 ± 1.0 vs. 9.9 ± 1.8 days in the control). Adult emergence was significantly reduced ($P < 0.0001$) in *Hypocrea* (21.4%) and *Beauveria* (38.0%) treatments as compared to the control (82.9%). The endophytic properties and the negative effects on host insect of tested isolates in this study warrant further research into their mode of action, development and use in the management of *L. huidobrensis.*

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

Overlapping gene functions in the endophytic insect-pathogenic fungus *Metarhizium*.

Israel Enrique Padilla-Guerrero[1], Zaizy Rocha-Pino[1,2], Keiko Shirai[2] and Michael J Bidochka[1].
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Metarhizium expresses a subset of genes as an insect pathogen or an endophyte and some of these genes are expressed under both conditions. We analyzed the previously published genome of *M. robertsii* and searched for genes expressed as a plant symbiont as well as an insect pathogen. Two genes, and their products, previously ascribed to insect pathogenesis also showed evidence for their utility during endophytism. We focused on a family of metalloproteinases that have a cellulose-binding domain (CBD1) and a chitinase-like protein (GH18) that inhibits xylanase. Since the time of divergence from the plant symbiont ancestor of *Epichloë* (ca. 100 million years ago), *Metarhizium* has also evolved as an insect pathogen. We suggest that certain genes used for endophytic ability have been evolutionarily co-opted for insect pathogenicity.

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

Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals plant pathogen-like effectors

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The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae and therefore has been seen as a potential biological control agent against disease vector mosquitoes. However, little is known about the pathological process of *L. giganteum* in its mosquito host. In order to detail the molecular basis of entomopathogenicity, Expressed Sequence Tags (EST) were generated using 454 pyrosequencing. To date, a total of 58,931 'Titanium chemistry' reads have been produced, and homology searches have led to the annotation of ca. 20,000 transcripts based on significant similarity to known proteins. A full complement of plant pathogenic oomycete effector orthologs was identified. Rapid Amplification of cDNA Ends (RACE) PCR reactions were used to obtain the full-length cDNA sequences of selected crinkler, elicitin and CBEL transcripts. Computational analyses predicted that the selected *L. giganteum* effector proteins are secreted and have similar domains than the *Phytophthora* spp. effectors, indicating that they may be involved in the pathogenicity process. In particular, the CBEL (Cellulose Binding Elicitor Lectin) orthologs contain the alternating Cellulose Binding and PAN/APPLE modules that have been associated with attachment to host tissue. The crinkler orthologs are characterized by a modular organization that includes the conserved LxLYLAR/K and HVLVxxP N-terminal motifs previously described for *Pythium ultimum* crinkler proteins. The roles of these effectors in the oomycete-insect host pathosystem are under investigation.

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

Characterization of a G-protein coupled receptor that links carbon sensing to conidiation, blastospore development, stress resistance, and virulence in *Beauveria bassiana*

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G-protein coupled receptors (GPCRs) act as sensors to link ligand and nutrient sensing to fungal development, morphogenesis, and where applicable, virulence. A GPCR was characterized from the entomopathogenic fungus, *Beauveria bassiana* (*BbGPCR3*) that links nutrient sensing to stress response and development. Loss of *BbGPCR3* resulted in reduced growth on various carbohydrates and glucose-specific developmental defects that included greatly reduced conidiation and blastospore development, effects suppressed in media containing trehalose or glycerol, but not by addition of cAMP. □*BbGPCR3* mutants were also impaired in their ability to respond to osmotic, oxidative, and cell wall stresses. In order to identify downstream targets of *BbGPCR3*, gene expression profiling of a set of heat-shock and antioxidant factors as well as compatible solute forming enzymes were examined after oxidative and osmotic stresses, respectively. These data revealed specific genes that failed to be induced in □*BbGPCR3* as compared to the wild type parent under each stress condition that could help account for the phenotypes observed. Insect bioassays revealed reduced virulence in topical assays but no effect in intrahemoceol injection assays, indicating that *BbGPCR3* was important in sensing signals during the initial interaction with the host but dispensable for post-penetration events.

Contributed paper. Monday, 15:30. **31 STU**

Cell wall integrity pathway regulates *Beauveria bassiana* responses to developmental and stressful cues via crosstalk with HOG pathway

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Cell-wall-integrity (CWI) pathway comprising Bck1, Mkk1 and Slt2 kinases controls yeast multi-stress responses via crosstalk with high-osmolarity-glycerol (HOG) pathway but such crosstalk has not been evident in filamentous entomopathogens. Sing-gene disruptions of *bck1*, *mkk1* and *slt2* in *Beauveria bassiana* caused drastic, but differential, defects in the fungal virulence and multi-stress responses and significantly less accumulation of intracellular mannitol and trehalose, but accelerated growth and conidiation on nutrition-rich medium, followed by faster loss of conidial viability during culture storage. Under cell-wall perturbing and hyperosmotic stresses, particularly, the phosphorylation levels of both Slt2 and Hog1 hallmarking the CWI and HOG pathways respectively decreased by 19□41% in the *Δbck1* and *Δmkk1* while *pbs2*, *ste11* and/or *sskB* upstream of *hog1* and other signaling genes were drastically repressed in *Δbck1*, *Δmkk1* and/or *Δslt2*. All the phenotypic changes were well restored by rescuing each disrupted gene.

Taken together, the CWI pathway interplayed mainly with HOG partners in regulating the fungal adaptation to diverse hosts and environments.

SYMPOSIUM (Viruses)
Monday, 16:30-18:30

Evolution of traits and host usage by the related polydnaviruses, baculoviurses, nudiviruses, and salivary gland hypotrophy viurses

Symposium. Monday, 16:30. **32**

TBD Just Vlak

Symposium. Monday, 16:50. **33**

Comparative genomics and evolution of baculoviruses, nudiviruses and bracoviruses.

Annie Bézier, Julien Thézé, Faustine Louis, Jean-Michel Drezen, Elisabeth A. Herniou
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Baculoviruses, nudiviruses and bracoviruses are related free-living and wasp symbiotic viruses that are pathogenic to invertebrate. Belonging to different virus families, they share common features but also display some specificity. Their packaged genome is made of single or polydispersed large circular dsDNA molecules, ranging from 80 to over 700 kb in cumulated size, and encoding from ~100 to 260 open reading frames with either viral or eukaryotic structure. All these viruses are produced within the nucleus of infected cells, or of wasp specialized ovarian cells, although their replication follow different models. Their rod-shaped enveloped nucleocapsids may be occluded or not. Comparative genomic studies revealed baculoviruses share 37 core genes and nudiviruses share 29 core genes including 20 of the baculovirus set. Eighteen of these core genes are involved in bracovirus production. These genes are mainly involved in replication, transcription, packaging/assembly and *per os* infectivity functions. Based on this data set we reconstructed the phylogenetic relationships of these viruses. As expected, baculoviruses are divided into four monophyletic genera. The nudiviruses form a sister group, which is more diversified within Arthropods and including two monophyletic lineages (OrNV/GbNV and HzNVs/PmNV/ToNV). Bracovirus evolved from a nudivirus ancestor belonging to the HzNVs/PmNV/ToNV clade, which integrated its genome within their common wasp ancestor about 100 million years ago.

Symposium. Monday, 17:00. **34**

Mutualistic Polydnaviruses Share Essential Replication Gene Functions with Pathogenic Ancestors

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Parasitoid wasps (order Hymenoptera) lay their eggs into other insects, where their progeny develop, feed, and ultimately kill their hosts. This creates an arms race between the wasps and the host's immune system. Wasps in the subfamily Braconidae have evolved an unusual weapon in their parasitism arsenal to suppress immune function: a symbiotic association with polydnaviruses in the genus Bracovirus (BV). Phylogenetic comparisons of viral genes indicate that BVs evolved from a pathogenic ancestor similar to nudiviruses and baculoviruses that was endogenized into wasp chromosomes approximately 100 million years ago. Pronounced differences in the biology of BVs and baculoviruses together with high divergence of many shared genes make it unclear whether BV homologs still retain baculovirus-like functions. Our results show that *Microplitis demolitor* bracovirus (MdBV) particles contain multiple baculovirus-like and nudivirus-like conserved gene products. Coupling RNAi knockdown methods with functional assays, we examined the activity of 6 genes in the MdBV conserved gene set that are known to have essential roles in transcription, capsid assembly, and envelope formation during baculovirus replication. Our results indicated that MdBV produces a baculovirus-like RNA polymerase that transcribes virus structural genes. Our results also supported a conserved role for *vp39, vlf-1, p74,* and *pif-1* as structural components of MdBV virions. Additional experiments suggested that *vlf-1* and the nudivirus-like gene *int-1* have novel functions in excision of MdBV proviral DNAs for packaging into virions. Overall, these data provide the first experimental insights linking BVs with other large DNA viruses that infect insects.

Symposium. Monday, 17:30. **35**

Functional Studies on the Hytrosaviridae: a large dsDNA Non-occluded Virus Infecting Adult Diptera

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The hytrosaviruses (SGHVS) are large, rod-shaped, dsDNA, enveloped viruses that infect adult dipterans and cause salivary gland hypertrophy (SGH). To date, majority of research has focused on the *Glossina pallidipes* SGHV measuring one micron in length (GpSGHV, 190-kbp genome, 160 putative ORFs) and the truncated *Musca domestica* SGHV measuring 600nm in length (MdSGHV, 124-kbp genome, 108 ORFs). The detection of SGH symptoms in the plant-feeding syrphid fly, *Merodon equestris,* and in the parasitic braconid wasp, *Diachasmimorpha longicaudata*, imply the family *Hytrosaviridae* contains other members. However the present lack of field-based insect virologists combined with the intrinsic properties of SGHVs (chronic infection, lack of overt symptoms, and infection of adult stage) has hindered their discovery. Although GpSGHV and MdSGHV show limited homologies in genome organization, sequence and proteome contents, the two viruses phylogenetically cluster together. Both viruses share certain properties: replication is limited to adult tissues (no replication in insect cell systems); nuclear-assembly and translocation of nucleocapsids via the nuclear pore; cytoplasmic virion envelopment; disintegration of cellular organelles; and continuous production and release of mature virions. Although GpSGHV and MdSGHV negatively impact adult fitness, reproduction, and mating behaviors in their respective hosts, there are distinct differences in the replication, pathology and transmission of the two hytrosaviruses. This presentation will provide an overview of the replication and impact of hytrosaviruses on their respective hosts, and wherever possible, review homologous and unique ORFs that mediate the pathobiology of hytrosaviruses.

Symposium. Monday, 17:50. **36**

A viral ancestor for the Virus-like particles of the ichneumonid wasp *Venturia canescens*

Apolline PICHON[1], Serge URBACH[3], Jean-Marc AURY[4], Annie BEZIER[2], Véronique JOUAN[1], Marc RAVALLEC[1], François WURMSER[2], Julie GUY[4], Valérie BARBE[4], François COUSSERANS[1], Jeremy GAUTHIER[2], Edith DEMETTRE[3], Vonnick SIBUT[2], Jean-Michel DREZEN[2], Anne-Nathalie VOLKOFF[1]
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Whereas many ichneumonid wasps rely on symbiotic polydnaviruses to successfully develop within their host, the *Campopleginae* wasp *Venturia canescens* relies on immune-suppressive Virus-Like-Particles (VLP) that are associated with the eggs (Rotheram, 1967). VcVLPs resemble viral particles but are devoid of DNA. Since their discovery, the question of their evolutionary origin has thrilled the scientific community. We have now deciphered the origin of these peculiar entities. They are not related to the well-known polydnaviruses carried by other related wasp species, but instead result from a third evolutive event of association between a pathogenic virus and a parasitic wasp, the third described in this complex group of parasitic wasps.

CONTRIBUTED PAPERS
Monday, 16:30-18:00

Microbial Control 1

Contributed paper. Monday, 16:30. **37**

***Drosophila suzukii*: searching for a microbial control**

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The spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is an important highly invasive pest of many soft fruit crops, including berries and grapes, that has recently established in both Europe and North America. The species is able to infest maturing, unharvested, as well as fallen fruit; consequently, grower *D. suzukii* control strategies rely on multiple chemical treatments over a large part of the growing season that will have long-term negative impacts on crop ecosystems. We screened the impact of multiple *Bacillus thuringiensis* serovars versus immature and adult *D. suzukii* and entomopathogenic fungi versus young adults. Under controlled conditions, several isolates were found to have a high impact on fly mortality.

Contributed paper. Monday, 16:45. **38**

Efficacy of a Cuban *Spodoptera frugiperda* MNPV in laboratory assays and preliminary field trials

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Spodoptera frugiperda attacks both maize and rice crops in Cuba and its control puts a heavy pesticide load on the environment as well as inviting resistance. This study was initiated to develop production processes and optimize field use of a Cuban isolate of SfMNPV to replace chemical pesticide use. The SfMNPV isolate used in this work was received by J. Ayala in 1995 from INISAV in Cuba. The lethal dose and time to death of the INISAV isolate was compared with that of other isolates from the collection of Dr. Jenny Cory, Simon Fraser University. It was among the most efficacious of the nine tested. Optimal dose and temperature to optimize mortality in 4th instar larvae were determined using leaf disk assays. Doses of 1x10^5 and 1x10^6 occlusion bodies (OBs) per larvae resulted in close to 100% mortality, five days post-infection. The optimal temperature for infection and efficacy was 29ºC, which was greater than that at 32ºC or 25ºC. Leaf disk assays combining boric acid (0.5 - 4%) with SfMNPV were fed to 4th instar larvae to determine an effect on efficacy. Observable effects were minimal perhaps because the quantity of boric acid delivered to the 4th instar larvae via the leaf disk was small. A preliminary field trial was conducted in maize in the spring of 2013 in Cuba in a field with a mixed larval population and 70% infestation. Seven days after the first application 30% of larvae were infected and after the second application 50% of larvae were infected. Our results suggest that this virus will be useful for pest management in sustainable maize production in Cuba.

Contributed paper. Monday, 17:00. **39**

Effectiveness of dsRNA versus siRNA in RNAi mediated gene knock-down in western corn rootworm (*Diabrotica virgifera virgifera*)

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Recent reports that RNAi can be used for in-plant control of western corn rootworm (WCR) have created an interest in understanding the RNAi mechanism in this important pest. In several insects, both long dsRNA precursors and processed siRNAs can cause knockdown of gene expression. In this report, we studied the effectiveness of dsRNA versus siRNA targeted against the V-ATPase subunit C gene in both WCR larvae and adult beetles. In 9-day diet feeding assays, dsRNA of at least 50-bp resulted in high levels of larval mortality. In contrast, 15-, 25-, or 27-bp dsRNAs and pooled 21-bp siRNAs caused no oral mortality of WCR larvae. Similarly, dsRNA caused 100 percent mortality of adult beetles while mortality of beetles exposed to siRNA was not different from negative control mortality. Further, when adult beetles were fed siRNA, there was no effect on the level of V-ATPase C mRNA at day 5, whereas WCR beetles fed with dsRNA exhibited ~35-fold reduction in V-ATPase C mRNA. Similar results were found with dsRNA/siRNA injections where we observed ~100-fold reduction in V-ATPase C mRNA level in beetles injected with dsRNA and no change in V-ATPase C mRNA in beetles injected with siRNA. Our results suggest that only longer dsRNA is effective in triggering knock down of V-ATPase C mRNA and causing WCR mortality. These results have implications for optimizing plant-delivered RNAi for rootworm control.

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

Malaria Mosquitoes Attracted by Fatal Fungus

Justin George[1], Nina E. Jenkins[1], Simon Blanford[1,2], Matthew B. Thomas[1,2], and Thomas C. Baker[1]
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Insect-killing fungi such as *Beauveria bassiana* are being evaluated as possible active ingredients for use in novel biopesticides against mosquito vectors that transmit malaria. Fungal pathogens infect through contact and so applications of spores to surfaces such as walls, nets, or other resting sites provide possible routes to infect mosquitoes in and around domestic dwellings. However, some insects can detect and actively avoid fungal spores to reduce infection risk. If true for mosquitoes, such behavior could render the biopesticide approach ineffective. Here we find that the spores of *B. bassiana* are highly attractive to females of *Anopheles stephensi*, a major anopheline mosquito vector of human malaria in Asia. We further find that *An. stephensi* females are preferentially attracted to dead and dying caterpillars infected with *B. bassiana*, landing on them and subsequently becoming infected with the fungus. Females are also preferentially attracted to cloth sprayed with oil-formulated *B. bassiana* spores, with 95% of the attracted females becoming infected after a one-minute visit on the cloth. This is the first report of an insect being attracted to a lethal fungal pathogen. The exact mechanisms involved in this behavior remain unclear. Nonetheless, our results indicate that biopesticidal formulations comprising *B. bassiana* spores will be conducive to attraction and on-source visitation by malaria vectors.

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

Studies on effect of spinetoram in *Helicoverpa armigera* (Hübner)

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The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the most important lepidoptern pests in China. Although most of *H. armigera* is effectively controlled by Bt cotton, but sometimes it still need using chemical insecticide sprays at 3rd and 4th generations in the field, at that time the expressed Cry1Ac toxic protein in cotton is low. But serious resistance of *H. armigera* to conventional insecticides has resulted in many problems. Spinetoram is naturally derived fermentation product from soil actinomycete *Saccharopolyspora spinosa*. Because of its unique action mechanism, spinetoram become the potential insecticide for management of resistance. In order to clarify the efficacy and mechanism of spinetoram, we assayed its effect on *H. armigera*. The spinetoram had good efficacy to control *H. armigera* larvae, the bioassay result showed LC_{50} was 1.23 ppm in susceptible strain. And it not only had lethal effects which decreased in survival of eggs, larvae and pupae, but also had sublethal effects which decreased in weights of larvae and pupae; prolonged periods of larvae, prepupae and pupae; shortened the adult longevity; decreased in the number of eggs laid per female. By the lethal and sublethal effects, spinetoralm changed the density and development of population. The detoxifying enzymes activity of *H. armigera* larvae could induce after treated by spinetoram at the tested concentration. Compared with control, the activities of EST, AchE and GST reduced, while the activity of MFO increased. Based on these facts, spinetoram showed its particular prospect in the future.

CONTRIBUTED PAPERS
Monday, 16:30-18:15

Fungi 2

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

Expression of *Bombyx mori* cecropin A in *Beauveria bassiana* ERL1170 to enhance mycotized mealworms for use as animal feed additives

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Antimicrobial peptides (AMPs) can be produced in mealworms, currently being used as animal feeds, by the infection of genetically engineered-entomopathogenic fungi. In this work, we integrated *Bombyx mori* (*Bm*) AMP, *cecropin A* to *Beauveria bassiana* ERL1170 by restriction enzyme-mediated integration method, which was confirmed by RT-PCR and an antibacterial activity assay. For the extracellular secretion of *Bm* cecropin A protein, the active domain of the *cecropin A* gene was tailed to the signal sequence of *B. bassiana* chitinase (*Bbs*). The *egfp* expression cassette including *gpdA* promoter and *trpC* terminator was cut from pBluescript II KS(+)-egfp using *Sac*I and *Cla*I and integrated into pBARKS1 containing phosphinothricin (PPT) resistant *bar* gene under the control of *trpC* promoter, designated as pBARKA1-egfp. To exchange *Bbs-cecropin A* gene with *egfp* gene in pBARKS1-egfp, *Bbs-cecropin A* fragment was cut from pGEM-Bbs-cecropin A using *Xba*I/blunted and *Bam*HI and ligated with cut pBARKS1-egfp using *Nco*I/blunted and *Bam*HI, designated as pBARKS1-Bbs-cecropin A. After the transformation, transformants were grown on Czapek's solution agar containing 600 μg ml^{-1} PPT. Expression of *Bm* cecropin A was confirmed by RT-PCR. Strong clear zone was observed in the co-culture of the transformant #37-21 and *Bacillus subtilis* on fourth strength Sabouraud dextrose agar 1 day after the culture at 25°C, whereas the wild type had no clear zone. This work suggests that *Bm* cecropin A can be efficiently produced in this mealworm-based fungal expression platform, thereby increasing the value of mealworms in the animal feed additive industry.

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

Protein and *Metarhizium*: are protein-loving locusts inadvertently increasing their chances of fungal infection?

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It is well established that the swarming behaviour of insects such as locusts is often driven by their need to ingest protein. Therefore, field locusts consume protein at every opportunity. Indeed, such insects often cannibalise one another to satiate their protein appetite, and it is this prospect of being cannibalised that drives individuals in the swarm ever onward. Using the Australian Plague Locust (*Chortoicetes terminifera*) as a model system, we were interested in examining how protein ingestion impacts host susceptibility to *Metarhizium acridum*, a fungal strain that is currently widely used as a commercial biocontrol product, GreenGuard®. We undertook some experiments showing that when fed a protein-rich artificial diet (35:7 ratio of Protein%:Carbohydrate%), locust mortality due to fungal infection was higher than when fed a balanced and carbohydrate-rich diet (21:21 and 7:35, respectively). Subsequent investigation of the host immune system indicated that immune-function increased when fed the protein rich diet. Although further investigation is required, we speculate that *Metarhizium* thrives in the protein-rich hemolymph environment, and therefore mortality is higher irrespective of increased host immune-function. This finding potentially has implications for the biological control of insect crop pests.

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

Effect of physiographic and climatic conditions on development of epizootics by *Entomophaga maimaiga* in outbreak gypsy moth populations

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Population outbreaks of the non-native gypsy moth, *Lymantria dispar*, often occur in periodic cycles, particularly in the US mid-Atlantic region. Outbreaking populations generally collapse after 1-3 years due to regulation by the fungus *Entomophaga maimaiga* and a nucleopolyhedrosis virus. Because *E. maimaiga* is sensitive to environmental and climatic conditions, we quantified the potential role of a suite of factors in driving *E. maimaiga* epizootics. We examined these dynamics at 63 mid-Atlantic sites during the final year of a gypsy moth outbreak. This work highlights the important role that physiographic conditions as well as microclimate play in gypsy moth outbreak regulation by *E. maimaiga*.

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

Bumblebee venom serine protease increases fungal insecticidal virulence by inducing insect melanization

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Insect-killing fungi have high potential for controlling agriculturally harmful pests. However, their pathogenicity is slow and this is one reason for their poor acceptance as a fungal insecticide. The expression of bumblebee, *Bombus ignitus*, venom serine protease (VSP) by *Beauveria bassiana* ERL1170 induced melanization of yellow spotted longicorn beetles, *Psacothea hilaris* as an over-reactive immune response, and caused substantially earlier mortality in beet armyworm, *Spodopetra exigua* larvae when compared to the wild type. No fungal outgrowth or sporulation was observed on the melanized insects, thus suggesting a self-restriction of the dispersal of the genetically modified fungus in the environment. The research is the first use of a multi-functional bumblebee VSP to significantly increase the speed of fungal pathogenicity, while minimizing the dispersal of the fungal transformant in the environment.

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

Using *Drosophila* as a model system for analyzing insect-fungal interactions

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In response to fungal infection, the insect innate immune system produces a group of antimicrobial peptides. Drosomycin is *Drosophila*'s prevalent antifungal peptide and homologs are produced by diverse insects. Its production in response to fungal attack is mainly regulated by the Toll immune pathway. We used a *Drosophila* strain with a drosomycin-GFP reporter to examine variation in *Drosophila*'s immune responses to fungi with different infection strategies, e.g., quick kill via toxins vs. slow kill via invasive growth. In addition, we used RNA interference (RNAi) to inhibit the function of immune genes in *Drosophila* and dissect their roles against the different fungi. Flies lacking the ability to produce *dif* (dorsal-related immunity factor) showed increased susceptibility to *Metarhizium* spp. The virulence of several *Metarhizium* strains was correlated with resistance to antifungal peptides. Flies defective in individual recognition proteins for fungal infections did not show altered responses to *Metarhizium* infection, suggesting that multiple detection mechanisms are required or sufficient to trigger immune pathway activation in response to fungal infection.

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

Characterization of a novel secreted insect toxic protein (Sit1) from the entomopathogenic fungus *Metarhizium anisopliae*

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Metarhizium anisopliae strain EAMa 01/58-Su secretes proteins that are toxic towards adult *C. capitata* and *Galleria mellonella* larvae. At least three proteins or peptides (mol wt. = 11, 15 and 15 KDa) were previously identified in culture supernatants as mediating insect toxicity. The active fractions were found to be toxic when injected into insect targets eliciting defense-related responses including melanization and tissue necrosis. Production of these proteins/peptides was confirmed both *in vitro* and during the infection process (*in vivo*). Two dimensional SDS-PAGE and mass spectrometry analysis indicated shared peptide sequences between the three proteins, suggesting that they were isoforms of the same protein, however no significant hits were identified in the mass spec protein databases used. Further bioinformatic analysis, however, was used to map the peptide fragments to a single *M. anisopliae* gene, which we propose to name secreted insect toxin-1 (*sit1*). A homolog of *sit1* was also isolated from the entomopathogenic fungus *Beauveria bassiana*. Sit1 was expressed and purified from a heterologous *E. coli* host. The purified recombinant protein displayed toxicity when injected into *G. mellonella* larvae. Mortality occurred within two days of injection and insect cadavers showed severe melanization responses.

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

Role of two Loss-of-aflatoxin expression (LaeA)-like putative methyltransferases in *Beauveria bassiana* development and virulence

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Fungal secondary metabolites are thought to function in diverse physiological processes ranging from environmental interactions to pathogenesis. The LaeA family of proteins encodes for putative methyltransferases that function as global regulators of gene expression originally isolated as regulating aflatoxin production in *Aspergillus* species. Two *LaeA* homologs, *BbLaeA* and *BbLaeB* were identified in *Beauveria bassiana* and the consequence of deletion of these genes was characterized. Loss of *BbLaeA* and □*BbLae*□*BbLaeB* double mutant strains resulted in defects in growth, conidiation, production of extracellular proteins, and expression of the hydrophobin gene. In contrast, □*BbLaeB* mutants were similar to wild type with respect to these phenotypes. Production of *B. bassiana* secondary metabolites, tennelin and oosporein, were altered in both □*BbLaeA* and □*BbLaeB* strains as compared to the wild type parent, although the effect was greater in the former mutant. Conidial viability was dramatically reduced in the □*BbLaeA* and □*BbLae*□*BbLaeB* mutant strains, and to a lesser degree for □*BbLaeB* mutants. Insect bioassays revealed that □*BbLaeA* and □*BbLae*□*BbLaeB* strains were attenuated in virulence, whereas □*BbLaeB* mutants displayed only a moderate reduction in pathogenesis. Constitutive expression of *BbLaeB* in a □*BbLaeA* background restored some but not all (most notably virulence) of the phenotypic effects seen in the mutant strain, indicating partial functional overlap between the two proteins.

TUESDAY - 13 August

SYMPOSIUM (Disease of Beneficial Invertebrates)
Tuesday, 08:00-10:00

Pathogens to control populations of invasive aquatic invertebrates

Symposium. Tuesday, 08:00. **50**

Challenges in microbial control of invasive mosquitoes and lessons learned

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Public health agencies are concerned with the establishment and spread of invasive mosquitoes in the US and their involvement in the transmission of pathogens to man and animals. Some established species include but are not limited to *Aedes aegypti*, *A. albopictus*, *A. japonicus*, and *Culex bahamensis*. Some of the pathogens of concern are those responsible for dengue, chikungunya, Japanese encephalitis and rift valley fever that have/could be introduced with the invasive mosquitoes. Integrated mosquito management programs include combining methods such as source reduction, pesticide applications and biological control to prevent transmission of vector borne pathogens. While *Bacillus thuringiensis israelensis* and *B. sphaericus* have been extremely successful in mosquito larval control they are not suitable for all species and habitats. Other possible agents include mermithid nematodes and microbes such as fungi, microsporidia and viruses. Examples from each of these groups will include *Romanomermis culicivorax* (mermithid), *Lagenidium gigantiem* (fungi), *Edhazardia aedis* (microsporidia) and CuniNPV (baculovirus). The mosquito species and habitats suited to control by each species will be discussed as well as issues and challenges relative to production, deployment, efficacy and safety.

Symposium. Tuesday, 08:20. **51**

Can aquatic invasive invertebrates be controlled by re-introduction to their native pathogens?

Stentiford, G.D.* and Stebbing, P.D.
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Field surveys of several ecologically- and economically-significant non-native invasive species (NNIS) of arthropod in the United Kingdom have revealed a relative lack of disease-causing pathogens known to occur within their native ranges. The most comprehensive study involved the so-called 'killer shrimp' *Dikerogammarus villosus*, first detected in UK fresh waters during 2010. The pathogen profile of *D. villosus* inhabiting the initial invasion site within the UK was compared to that in two other invasive populations within the UK, to two sites within the European continental-invasive range in France and Poland, and to those pathogens known to occur in *D. villosus* within their native range (Ponto-Caspian region). The cumulative data revealed that although some commensal organisms were shared between native and invasive populations, several important pathogens present in the continental-invasive and native ranges, were absent from UK populations. In particular, it is proposed that the absence of pathogenic microsporidian parasites (such as *Cucumispora dikerogammari*) and a novel virus (*Dikerogammarus villosus* bacilliform virus) from the UK, likely impart significant survival advantages to *D. villosus* over native fauna in the invasive range, thereby increasing their success as invaders. In essence, the contrast in pathogen profile between UK and continental-invasive populations of *D. villosus* provides strong evidence for 'enemy release' in these island populations and may reflect single-point, rather than continual incursion events in the case of the UK invasion. Similar examples exist for UK populations of the Chinese mitten crab (*Eriocheir sinensis*) and the signal crayfish (*Pacifastacus leniusculus*) – both considered as high profile NNIS in Europe. In this presentation, I will discuss these case studies in relation to the desire to limit the impact of NNIS in Europe and the potential for bridging the gap between integrated pest management strategies widely applied in terrestrial systems, and the control of aquatic arthropod pests.

Symposium. Tuesday, 08:40. **52**

Exploration of potential microbial control agents for the invasive crayfish, *Orconectes virilis*

Elizabeth W. Davidson[1], Jennifer Snyder[2], Donald Lightner[3], Gregory Ruthig[4] Julie Lucas[1] and Joel Gilley[1]
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The introduced crayfish, *Orconectes virilis*, has become a serious invasive species in Arizona (USA), altering stream ecosystems and contributing to the decline of native species. But because it is native to eastern US, and related crayfish including endangered species inhabit nearby states, introduction of a biological control agent presents a unique challenge. We explored 12 potential bacteria, nematodes and viruses for control of *O. virilis.* Only White Spot Syndrome Virus (WSSV), a pathogen of shrimp, was found to be highly pathogenic and was readily passed by cannibalistic behavior but not by water transmission. WSSV is an OIE (World Organization for Animal Health) listed disease of major concern to the shrimp and commercial crayfish industries (OIE 2003), and therefore we could not propose release into the field. This experiment exemplifies problems that may arise with attempts to find acceptable biocontrol techniques for invasive aquatic species.

Symposium. Tuesday, 09:00. **53**

Microbial control of an invasive aquatic mollusc, the zebra mussel – The quest from the researcher's perspective

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It's easy to come up with a hypothesis. As every researcher knows, proving it can be another matter. Back in 1991 when zebra mussels had just started to spread into New York State and to foul power plant intake pipes, the New York State Department of Environmental Conservation approached my lab inquiring whether a biological control technique could be developed. In response, I came up with a hypothesis -- a control idea that grew out of my years of research on Bti as a black fly biocontrol agent. I predicted that with proper funding my lab could isolate a bacterial strain in nature that would not only be lethally intoxicating to these mussels, but also highly host specific to them. Little did I know that it would take two decades of intense R&D at my lab and over five million dollars in extramural funding to turn that control concept -- that control dream -- into a commercial reality. This is the story of the project's rollercoaster research journey, starting with years of failed screening trials, a breakthrough discovery of a novel toxic strain of the soil bacterium *Pseudomonas fluorescens*, the challenges of fermentation scale-up, and the years of efficacy and non-target trials that culminated in the licensing of this bacterial strain as a zebra mussel biopesticide under the product name Zequanox®. It doesn't happen very often when doing research on biopesticide development, but this time a dream really did come true.

Symposium. Tuesday, 09:20. **54**

Development of a Microbial Control for Invasive Quagga and Zebra Mussels

Carolyn Link, MSc, Sarahann Rackl, Ph.D., P.E., Pam Marrone, Ph.D.

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Invasive zebra and quagga mussel populations (*Dreissena* species) are a prevalent problem throughout European and North American waters. These prolific filter feeders are causing economical and ecological damage to freshwater reservoirs, waterways, and infrastructure with limited available tools for control. In ecosystems where invasive mussels are present, the abundance of native organisms decrease dramatically while growth of unwanted weeds and algae increase. This ecological imbalance can negatively impact fisheries, recreation, irrigation lines, and industrial service water systems. Current chemical mussel control methods can provide efficacy, but come with their own economic and ecologic implications. Zequanox®, an environmentally compatible microbial molluscicide developed by Marrone Bio Innovations, Inc., is used to control zebra and quagga mussels without harming humans, infrastructure, non-target species, or the environment. The active ingredient in Zequanox is killed cells from a ubiquitous soil microbe—*Pseudomonas fluorescens*. Toxicology studies demonstrate that the product is highly selective toward *Dreissena* mussels; at concentrations that produce mussel mortality, no product-induced mortality was recorded among non-targets including algae, fish, mollusks, or crustaceans. Zequanox is registered for use in industrial closed or semi-closed systems (e.g., cooling water systems) and can now be used as an alternative to chemicals like chlorine and quaternary ammonium compounds. This presentation will review the discovery and development of the product, including recent research into application patterns for reducing mussel settlement and controlling veligers. We will also summarize treatments conducted at facilities in the U.S., Canada, and Europe, and discuss the process towards expanding use of the product internationally.

CONTRIBUTED PAPERS
Tuesday, 08:00-10:00

Fungi 3

Contributed papers. Tuesday, 08:00. **55 STU**

Investigating Asian longhorned beetle immunity following maternal immunepriming with a fungal pathogen

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Insects have an innate immune system that, although having the ability to respond quickly and efficiently to many types of microbial and parasitic attacks, is thought to lack the ability to acquire immunity through memory. It has been demonstrated that in several insect species, including *Tenebrio molitor* and *Tribolium castaneum*, parental exposure to a bacterial pathogen increased offspring immunity. Little work has been done however to determine whether parental priming with a fungal pathogen increases offspring immunity or if trans-generational immune priming occurs in other beetle taxa. Asian longhorned beetles (ALB), *Anoplophora glabripennis*, from China are invasive woodborers in the eastern United States with the potential to negatively impact economic and environmental interests in US hardwood forests. The entomopathogenic fungus *Metarhizium brunneum* has been shown to be effective in killing ALB but it is unknown whether exposing beetles to a sub-lethal fungal dose will affect offspring immunity. Adult female beetles were primed with one of the following treatments: live *Metarhizium brunneum*, heat-killed *M. brunneum*, live *M. anisopliae*, heat-killed *Serratia marcescens*, control treatments. One-week post priming, beetles were allowed to mate and lay eggs. The adult offspring of primed beetles were then challenged with a lethal dose of *M. brunneum*. Bioassays were conducted to determine the impact of immune priming on beetle longevity and the cellular immune response was quantified 14-16 days post inoculation. To determine whether there was a fitness cost associated with maternal priming successful survival to pupation and adult weight of offspring from primed females were quantified.

Contributed paper. Tuesday, 08:15. **56**

Occurrence of *Metarhizium anisopliae* in an organic, rotational no-till cropping system

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Relatively few pest management materials are allowed in organic crop production; therefore, transitioning and organic farmers must largely rely on cultural practices and biological processes to manage pests. We examined the effects of sequential management to reduce weed and insect pest impacts on naturally-occurring entomopathogenic fungi (EPF) in an organically-managed no-till planted maize (*Zea mays*), soybean (*Glycine max*), and conventionally-tilled winter wheat (*Triticum aestivum*) rotation. Management practices include three planting dates for maize and soybean; over-wintering cover crops terminated using a roller-crimper followed by no-till planting of maize and soybean; and supplemental weed management in maize and soybean using shallow high-residue cultivation. Overwintering cover crops, established using inversion tillage, include a hairy vetch (*Vicia villosa* Roth.)/triticale(*Triticosecale*) mixture preceding maize, and cereal rye (*Secale cereal*) preceding soybean. We detected two species of EPF *(Metarhizium anisopliae* (Metschn.) Sorokin, *Beauveria bassiana* (Bals.-Criv.) Vuill. by bioassay of soil samples collected twice during each field season. Detection of *M. anisopliae* varied across crop species and year in crop sequence. No consistent trend was observed according to planting date. We detected *M. anisopliae* more frequently in 2011 and 2012 compared to 2010, when the experiment was initiated, and more frequently in maize than in soybean and wheat. Detection was greater in plots receiving two compared with no or one tillage event. Analysis of relationship of *M. anisopliae* detection with other soil chemical and physical characteristics is in progress. This study provides information about management effects on soil function, specifically conservation biological control.

Keywords: *Metarhizium anisopliae*, conservation tillage, cover cropping, conservation biological control, organic production

Contributed paper. Tuesday, 08:30. **57**

Laboratory and greenhouse evaluation of a new entomopathogenic strain of *Beauveria bassiana* for control of the onion thrips *Thrips tabaci*

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The onion thrips *Thrips tabaci* is one of the most important pests of greenhouse and open-field broccoli, onion and other crops. However, the current strategy of using synthetic pesticides for its control is inadequate and unsustainable, leading to a growing interest in novel and effective biological control alternatives such as entomopathogenic fungi. The most important findings were strain SZ-26 was found as the most potent after a laboratory screen of 20 new entomopathogenic strains of *Beauveria bassiana* against *T. tabaci*, causing 83-100% mortality in adults at 1×10^7 mL^{-1}conidia after 4-7 days. Further experiments in greenhouses showed the strain SZ-26 significantly lowered the numbers of adult and larval stages. In conclusion, this project demonstrated the biocontrol potential of the new *B. bassiana* SZ-26 strain and that further product development work needs to be conducted to support the use of this fungus-based biopesticide for control of *T. tabaci* in broccoli and onion agroecosystem.

Contributed paper. Tuesday, 08:45. **58**

Investigating Metarhizium brunneum F52 microsclerotia applied as a hydromulch for the potential biological control of Asian long horned beetle, Anoplophora glabripennis

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The use of Metarhizium brunneum F52 microsclerotia formulated with diatomaceous earth and clay carriers and incorporated into hydromulchs and sprayed onto trees, is an application strategy which may hold promise for effective biological control of A. glabripennis. Microsclerotia are compact, heavily melanised, hyphal aggregates which are usually produced under specific growth conditions in liquid media and offer an environmentally persistent form of this fungus. Concurrently, hydromulching, which conventionally involves spraying a mixture of water, fibre mulch (hydrostraw) and a tackifier onto burned slopes to prevent soil erosion, has not yet been investigated with microsclerotia in an aerial arena for pest control. Preliminary research, using 0.35 g of hydromulch with 4% guar tackifier, 0.13 g of microsclerotia and 2 ml of water, applied to water agar, filter paper, moist bark and dry bark pieces showed that microsclerotia germinated and produced significantly more conidia on water agar, filter paper and moist bark. However, there was a delay in conidia production on dry bark, highlighting the importance of moisture for growth of microsclerotia. When A. glabripennis beetles were exposed to high (0.35 g) and low (0.13 g) treatments of hydromulch plus 0.13 g of microsclerotia and 2 ml of water, applied to filter paper and dry bark pieces, it took significantly longer for beetles to die when exposed to drier bark pieces compared to treated filter paper. Median survival times for filter paper with low and high hydromulch treatments were 8 and 9 days respectively, and for dry bark pieces with low and high hydromulch treatments, 17.5 d and 16.5 days respectively. Future studies will evaluate the oviposition preference, fecundity and survival of A. glabripennis on logs sprayed with hydromulch and incorporated microsclerotia.

Contributed paper. Tuesday, 09:00. **59**

***Beauveria bassiana* as a potential agents for emerald ash borer management: Tracking tool to monitor a post-release isolate.**

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The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), is an invasive wood boring beetle that is destroying North America's ash trees (*Fraxinus* spp.). *Beauveria* spp. isolates were recovered from dead and mycosed EAB cadavers from outbreak sites in southern Ontario, Canada. Molecular characterization using sequences of the internal transcribed spacer (ITS), 5' end of elongation factor 1 alpha (EF1-α) and intergenic Bloc loci revealed that *Beauveria bassiana* and *Beauveria pseudobassiana* were commonly associated with EAB in the sampled sites. Virulence screening against EAB adults using 24 *Beauveria* spp. isolates and compared with the commercial isolate, GHA revealed that the indigenous *B. bassiana* isolate, L49-1AA was significantly more virulent and produced more conidia on EAB cadavers than all tested isolates. An auto-contamination trapping system have been designed and implemented to disseminate L49-1AA in the field to manage EAB populations. We have developed a simplified allele discrimination polymerase chain reaction (PCR) assay based on allelic inhibition of displacement activity (AIDA) for the discrimination of *B. bassiana* L49-1AA from all background *Beauveria* isolates. The designed biomarker is being used to monitor the introduced L49-1AA in terms of its establishment, transmission and persistence in the environment.

Contributed paper. Tuesday, 09:15. **60**

The effect of *Neozygites floridana* killed *Tetranychus urticae* females on sexual behavior of *T. urticae* males

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The entomopathogenic fungus *Neozygites floridana* (Neozygitomycetes: Neozygitales: Neozygitaceae) infects and kills the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). In addition to the resting spore stage, the *N. floridana* killed *T. urticae* cadaver appears in three different "stages": 1) Fresh non-sporulating cadaver 2) Sporulating cadaver that produces primary conidia 3) Sporulating cadaver that produces secondary (capilli) conidia. It is only the secondary (capilli) conidia that may result in infections of *T. urticae*. The effects of these three "cadaver stages" on sexual behavior of *T. urticae* males was tested in a series of experiments. In the first experiment *T. urticae* males were exposed to two non-moving *T. urticae* females, namely a healthy quiescent deutonymph and a *N. floridana* killed *T. urticae* cadaver in one of the three "cadaver stages" mentioned above. The following behavior was recorded: 1) In direction of which female did the male move (choice)? 2) How frequent did the male touch the different females? 3) How frequent did the male guard the different females? Results suggest that non-sporulating female cadavers are more attractive than healthy females to *T. urticae* males. Further, the "cadaver stage" significantly affected the *T. urticae* male choice, touching and guarding behavior. To reveal whether a similar attraction were seen to *N. floridana* killed *T, urticae* males, a second experiment, where healthy *T. urticae* males could choose between a non-sporulating male cadaver and a non-sporulating female cadaver, was conducted. Results showed that *T. urticae* males were more attracted to female cadavers than male cadavers.

Contributed paper. Tuesday, 09:30. **61 STU**

***Neozygites osornensis* sp. nov., a new fungal species causing mortality to the cypress aphid *Cinara cupressi* in Chile**

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An entomophthoroid fungus causing epizootics in populations of the cypress aphid, *Cinara cupressi* Buckton, in Chile is described as a new species, *Neozygites osornensis* Montalva et Barta. The new aphid pathogen is described based on morphological characters and molecular analysis. An exhaustive description, illustrations and a comparison with closely related species are provided. The fungus differs from similar *Neozygites* species by smaller size of hyphal bodies, nuclei, primary conidia, capilliconidia and capilliphores and by noticeably different shape of capilliconidia. Also a partial sequence of 18S rDNA allowed separating among the morphologically similar and related species *Neozygites cinarae*, *Neozygites turbinata* and *Neozygites osornensis*. A *in silico* RFLP and phylogenic analysis were included to support our results.

Contributed paper. Tuesday, 09:45. **62**

Microbial control of the Asian ambrosia beetle *Xylosandrus germanus*

John Vandenberg[1], Louela Castrillo[2] and Michael Griggs[1]
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The ambrosia beetle *Xylosandrus germanus* is a widely-distributed and well-established invasive insect with a wide host range. It is a serious pest of orchards and nurseries in the eastern and midwestern US and is difficult to control using conventional insecticides. We evaluated a commercial formulation of *Metarhizium brunneum* by treating logs of American beech in a forest in Tompkins County NY. Beetle attack rate was significantly reduced versus controls. Survival of foundress females was reduced among those attacking *M. brunneum*-treated logs. Fewer galleries and fewer offspring were present in fungus-treated logs. Infection of adults, larvae and pupae by *M. brunneum* was evident with galleries. Infection of adults, larvae and pupae by M. brunneum was evident within galleries. These results indicate the potential utility of this biopesticide for managing Asian ambrosia beetles.

CONTRIBUTED PAPERS
Tuesday, 08:00-09:45

Bacteria 2

Contributed paper. Tuesday, 08:00. **63**

Novel protein production system using a peptide-tag derived from *Bacillus thuringiensis* mosquitocidal Cry4Aa toxin.

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Cry4Aa is a dipteran-specific Cry toxin produced by *Bacillus thuringiensis* subsp. *israelensis*, and is accumulated in the form of protein crystals. This crystal formation is considered as a nice strategy to produce protein efficiently. Crystal formation allows a large amount of protein to be packed into the limited intracellular space, and protects protein from proteolitic degradation in the environment. We have developed a novel system to prepare proteins efficiently by using peptide-tag derived from the mosquitocidal Cry4Aa toxin. Fusion with this peptide-tag, designated 4AaCter, facilitates the formation of crystal-like inclusion bodies of heterologous proteins in *Escherichia coli*. To evaluate the usability, the 4AaCter-tag was applied for production of recombinant proteins in high demand for diagnosis purpose. Application of 4AaCter-tag to the production of syphilis antigens (TpNs) from *Treponema pallidum* yielded excellent results, including a dramatic increase in the production level, simplification of the product purification and high reactivity with syphilis antibody. Similarly, fusion with 4AaCter-tag increased the production level of human cystatin C, and highly purified protein was obtained without the need for complicated purification steps. The recombinant cystatin C showed high immunogenicity and immunoreactivity as that of native human cystatin C. We believe these experiments can be milestones to evaluate the usability of 4AaCter-tag, and our results suggested that the protein production system using 4AaCter-tag could be a powerful mean of preparing significant amounts of antigen protein.

Contributed paper. Tuesday, 08:15. **64**

The novel gene discovery of *Bacillus thuringiensis* in northeast region, China

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Bacillus thuringiensis (Bt) is the most widely used as insecticidal microbes. One hundred and eighty-nine Bt isolates were screened from 1400 soil samples collected from primeval forests in Northeast China.Sixty-nine isolates harbored *cry1* genes, 29 isolates harbored *cry2* genes, 5 isolates harbored *cry7* genes, 9 isolates harbored *cry8* genes. A new polymerase chain reaction–restriction fragment length polymorphism method for the identification of cry8-type genes from *Bacillus thuringiensis* has been established. Bt strains QZL26, QWH183, QWH108, QZL85, QZL1-2 from Liaoning Qianshan containing *cry8* genes respectively. According to known full-length gene sequence,designe full-length primers, successful cloned the genes. *cry8Da*, *cry8Fa*, *cry8Ka* and three *cry8Ca*, the new genes were cloned into a heterologous expression. By this method, a novel gene, *cry8Na1*, encoding a polypeptide of 1,157 amino acids was identified and cloned from Bt Q52-7. Recombinant Bt strain HD8N, harboring *cry8Na1*, has insecticidal activity against larvae of Melolonthidae pests: *Holotrichia parallela*. The protein to *Holotrichia parallela* has obvious biological activity, and LC_{50} 0.818×10^{12} cfu / g, 95% confidence interval is from 0.373×10^{12} to 1.742×10^{12} cfu / g. Meantime, to further explore *Bacillus thuringiensis* strains and the novel gene resources, we get a 1038 bp DNA sequence from QZL26 isolate which codes 345 amino acids and the similarity of the amino acid sequence with the Sip1A is 91.83%. Sequencing result was submitted to GenBank, and the registration number is JQ965994

Contributed paper. Tuesday, 08:30. **65**

Regulation and roles of "host iron" acquisition systems in *Bacillus cereus* & *B. thuringiensis* during infection

Diego Segond[1], Elise Abi Khalil[1], Christophe Buisson[1], Ahmed Yakoubi[1], Amani Farhat[1], Mireille Kallassy and Christina Nielsen-LeRoux [1]
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The ability of *B. cereus* and B. *thuringiensis* to colonize various mammals and insects is linked to the presence of several adaptation factors, one of which is the capacity to acquire iron. Previously, an *in vivo* screen of *B. cereus* led to the identification of a novel protein, IlsA, which is specifically expressed in the insect hemocoel and under iron restrictive conditions *in vitro*. It was further shown that IlsA is localized on the surface of *B. cereus* and affinity tests revealed that IlsA interacts with both hemoglobin and host ferritin. Inactivation of *ilsA* decreases virulence in an insect model *Galleria mellonella* (PloS Pathogens, 2009 (11) e1000675). We have recently shown (submitted for publication) that IlsA interferes with another surface protein, Isd (Iron surface determinants), to insure heme uptake and that the *B. cereus* siderophores (bacillibactin) is particularly important for ferrtine iron uptake and for full virulence in *G. mellonella*. Next we aimed to determine the relative role of the major transcriptional regulators, Fur and CodY, in the expression of these three (IlsA, siderophores and Isd) iron acquisition systems and to elucidate when and where the systems are expressed during infection. For that purpose plasmid-born transcriptional *lacz*-promoter-fusions were constructed and transformed into *B. cereus* ATCC14579 wild type strain and the Δfur (iron uptake repressor) and Δ cody mutants. Expression was studied *in vitro*, in iron rich and iron depleted medium and in *Galleria* larvae, in bacteria isolated from the midgut and from the hemocoel. The presentation will mainly focus on results from gene expression studies.

Contributed paper. Tuesday, 08:45. **66**

Molecular Genetic Basis for Engineering Small to Large Crystals of Cyt1Aa

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The parasporal body of the mosquitocidal bacterium, *Bacillus thuringiensis* subsp. *israelensis*, consists of four major crystal proteins, Cry4Aa, Cry4Ba, Cry11Aa and Cyt1Aa, surrounded by a fibrous matrix of undefined composition. Within the parasporal body, the Cyt1Aa crystal is the largest, making up 55% of its dry weight. In previous studies we showed that *cyt1Aa*

expression is under the control of three strong sporulation-dependent promoters. By evaluating the levels of Cyt1A synthesized by these alone and in permuted combinations, giving the level of synthesis obtained with the weakest promoter a value of 1, we were able to obtain crystals that varied in size from 1 – 4-fold. More recently, using all three promoters and various expression vectors in which the origins of replication and selectable markers were altered, we were able to increase our levels of synthesis over the above by approximately 2-fold to a value of 8. These results show that using *cyt1a* promoters alone and in combination with other molecular genetic traits, a wide range of levels of Cyt1A synthesis, and likely other endotoxin proteins, can be obtained using *Bacillus thuringiensis* and other bacillus species as expression vectors. These molecular genetic strategies provide the possibility of constructing more efficacious strains of bacterial insecticides targeted against specific pest species using defined amounts and ratios of different endotoxins.

Contributed paper. Tuesday, 09:00. **67**

The toxins and their synergy in Bacillus thuringiensis strain HBF-18

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Scarabaeoidea is the largest group of subterranean pests and resulting in the most loss. *B. thuringiensis* strain HBF-18 was reported toxic to *Holotrichia oblita* and *H. parallela*. From the HBF-18, the prior cloned *cry8Ga1* gene was toxic to *H. oblita* and *H. parallela*, but the toxicity (HD8G) was significantly lower than that of HBF-18. After genome sequencing and toxin gene scan, three novel genes (*cry8like*, *vip1like* and *vip2like*) were detected and cloned. Recombinant *B. thuringiensis* strains HD8X, HDVip1 and HDVip2, harboring the novel gene respectively, have significantly higher toxicity than that of HD8G to *H. oblita* and *H. parallela*. In addition, HD8X synergizes HD8G (express Cry8Ga1 protein) with synergistic factor (SF) was 20.2 for *H. oblita*, 5.5 for *H. parallela*, and 6.0 for *Anomala corpulenta*, whilst Cry8X and binary of Vip1-Vip2 synergizes Cry8Ga1 with SF was 3.1, 12.5 and 54.8 for the three pests respectively. RT-PCR analysis indicated that the *cry8like*, *vip1like* and *vip2Alike* genes could be transcribed in HBF-18 and it suggested that the synergistic effect of the *cry* and *vip* genes leading to high toxicity of *B. thuringiensis* strain HBF-18 against Scarabaeidae larvae.

Contributed paper. Tuesday, 09:15. **68**

Screening of *cry*, *vip* and *cyt* genes in *Bacillus thuringiensis* strains collected from the Amazon biome in Brazil.

[2]André Mourão; [2]Arthur Torres; [1]Rosane Silva; ; [3]Fernando Valicente
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Bacillus thuringiensis (Bt) is a gram-positive bacterium that can be found in soil, grain dust and water from different ecosystems. In the search for new genes, PCR has been the most used technique. A total of 72 soil samples were collected from three different ecosystems in the Amazon basin, during the raining season as follows: 24 samples from annual cropping system (AAA), 24 from perennial cropping system (AAP), and 24 from native vegetation (VN). Samples for each ecosystem were at least 2km away. A total of 474 colonies were isolated and 265 were checked as Bt. From all 265 colonies, 29.43% of the Bt were present in the AAA cropping system, 43.77% in AAP cropping system, and 26.8% in the VN. Molecular characterizations were PCR based using specific primers for *cry* genes *(cry1Aa/cry1Ad, cry1Ab/cry1Ac, cry 1C, cry1Ea/cry1Eb, cry1Fa/cry1Fb, cry1Fa1/cry1Fb, cry 1I, cry 2Aa, cry 2Ab, cry2Ac, cry9B), vip* primers *(vip1, vip2, vip3, vip3Aa1, vip3Ah1, vip3Ae1, vip3Ba1, vip3Aa2, vip3Af1),* and *cyt* primers *(cyt1, cyt1Ab, cyt1Aa, cyt2Ba).* 22 gene amplifications were found it the Amazon basin biome. AAPerennial showed the highest number of genes (50%), and only 22.7% of the strains in the VNative. *cry1I* was the most frequent gene and appeared in 6 strains, followed by and *vip2* gene in 4 strains. *cry1Ab/cry1Ac, cry1C, cry1Fa1/cry1Fb, cry2Aa, cry2Ac, cry9B, vip1, vip3Aa1, vip3Ae1, vip3Ba1, vip3Aa2, vip3Af1, cyt1Ab* and *cyt1* did not amplified in any of the samples.

Contributed paper. Tuesday, 09:30. **69**

c*ry, vip* and *cyt* genes occurrence in *Bacillus thuringiensis* strains isolated from the Cerrado region in Western Central in Brazil.

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Bacillus thuringiensis (Bt) is an ubiquitous bacterium that can be isolated from different ecosystems. In the search for new genes, PCR has been distinguished by its detection level, easy to use and repeatability of results. A total of 72 soil samples were collected from three different ecosystems in the Cerrado area during the raining season as follows: 24 samples from annual cropping system, 24 from perennial cropping, and 24 from native vegetation. Samples for each ecosystem were at least 2km away. A total of 521 colonies were isolated and 354 were detected as Bt. From all 354 Bt colonies, 46.61% of the Bt, were present in the annual cropping area, 27.4% in the perennial cropping area, and 25.98% in the native vegetation. Molecular characterizations were PCR based using specific primers for *cry* genes *(cry1Aa/cry1Ad, cry1Ab/cry1Ac, cry 1C, cry1Ea/cry1Eb, cry1Fa/cry1Fb, cry1Fa1/cry1Fb, cry 1I, cry 2Aa, cry 2Ab, cry2Ac, cry9B), vip* primers *(vip1, vip2, vip3, vip3Aa1, vip3Ah1, vip3Ae1, vip3Ba1, vip3Aa2, vip3Af1),* and *cyt* primers *(cyt1, cyt1Ab, cyt1Aa, cyt2Ba).* 48 gene amplifications were found in the Cerrado biome. Perennial and annual cropping area showed the highest number of genes (37.5%), and the native vegetation showed the lowest number of genes (25%). *vip2* gene and *cry1I* gene were the most frequent and appeared in 12 strains, followed by *cry1Aa/cry1Ad* gene (5 strains). *cry1C, cry1Fa/cry1Fb, cry2Ac, cry9B, vip3Ae1, vip3Ba1, vip3Aa2, cyt1, cyt1Aa, cyt1Ab* and *cyt2Ba* did not amplified in any of the samples.

CONTRIBUTED PAPERS
Tuesday, 08:00-10:00

Nematodes 2

Contributed papers. Tuesday, 08:00. **70**

The response of *Caenorhabditis elegans* to bacteria from its natural environment

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As a natural bacterivore, the nematode *Caenorhabditis elegans* is a tractable system in which to investigate interactions between bacteria and the eukaryotic digestive tract. This system has already yielded insights into the molecular crosstalk between medically relevant pathogens and the host intestine. However, until recent work demonstrated that wild *C. elegans* populations are often found in association with rotting fruit, it was unclear what types of microbial communities would be naturally encountered by *C. elegans*, and what sorts of beneficial or detrimental interactions might occur between *C. elegans* and bacteria in the wild. We are beginning to address these questions using a panel of bacterial isolates that were collected by Dr. M-A Félix at seven microsites in France (e.g. one rotting apple) where *C. elegans* populations were also found. The isolates have been assigned to 18

genera on the basis of 16S rDNA sequencing. Our hypothesis is that this collection of bacterial isolates includes both potential pathogens and potentially beneficial microbes, able to protect nematodes from pathogens by colonizing the intestine or by priming the immune system. We will show that the isolates vary both in their effect on the lifespan of adult animals, and in their capacity to elicit immune responses such as induction of putative antimicrobial genes. Finally, we will present evidence that nematode immune pathways characterized in the context of the response to human pathogens also play a role in responding to these naturally isolated bacteria.

Contributed papers. Tuesday, 08:15. **71**

Biological control potential of five Turkish entomopathogenic nematode isolates against the lawn caterpillar, *Spodoptera cilium*

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Spodoptera cilium, known variously as lawn caterpillar, dark mottled willow, and grasslawn armyworm, is a noctuid moth found throughout much of Africa, Asia and Europe. Lawn caterpillar generally attacks lawns, turf and rice. Recently, turf areas of tourist hotels in Sarigerme town of Mugla, Turkey had a serious problem with *S. cilium*. Because of human health issues, chemical pesticides could not be used around the hotels. Therefore, an alternative control tactic was needed for the control of lawn armyworm larvae. Our objectives which targeted the larval stage of *S. cilium* were to (1) evaluate the biological control potential of five native entomopathogenic nematode (EPN) species, and (2) test the efficiency of selected EPN species in a semi-field experiment. Although all five native species provided 100% mortality of *S. cilium* larvae in laboratory tests, we selected *Steinernema carpocapsae* and *H. bacteriophora* (Sarigerme) for further semi-field studies because of their foraging strategy. *S. carpocapsae* and *H. bacteriophora* averaged 77 and 29% larval mortality, respectively, whereas control mortality was 1.6%. **Key words**: *Spodoptera cilium*, Entomopathogenic nematode, *Steinernema*, *Heterorhabditis*, Biological control

Contributed papers. Tuesday, 08:30. **72**

Does Combining Entomopathogenic Nematode Species Enhance Control of Curculio elephans (Coleoptera, Curculionidae) Overwintering Larvae?

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The larva of Curculio elephas (Coleoptera: Curculionidae) is a key pest of chestnut fruit. Because the last larval stage overwinters in the soil, it is an excellent candidate for control using entomopathogenic nematodes. However, our previous research has shown that this insect is relatively resistant to nematode infection with less than 50% larval mortality using Steinernema feltiae, S. weiseri or Heterorhabditis bacteriophora. We hypothesized that a combination of different entomopathogenic nematode species could enhance efficiency against this chestnut pest. Accordingly, we evaluated the efficacy of S. glaseri, S. weiseri or H. bacteriophora alone or in combination using 24-well tissue culture plates filled with 0.5 g sterilized sandy soil. Our results showed that S. glaseri, S. weiseri and H. bacteriophora alone provided 21, 50 and 64% larval mortality, respectively. With a combination of two species, S. glaseri + S. weiseri showed 68%, S .glaseri + H. bacteriophora resulted in 46%, and S. weiseri + H. bacteriophora gave 71% larval mortality. The triple combination of S. glaseri + S. weiseri + H. bacteriophora resulted in 68% larval mortality. Combination of S. glaseri + S. weiseri showed synergistic effect whereas, S .glaseri + H. bacteriophora and S. weiseri + H. bacteriophora showed antagonistic effect.

Contributed papers. Tuesday, 08:45. **73 STU**

Nematicidal activity of Crude Extracts of the Entomopathogenic Bacterium, *Photorhabdus l. sonorensis* on the Root-knot Nematode (*Meloydogyne incognita*) and the Stem Gall Nematode (*Anguina pacificae*)

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Entomopathogenic bacteria in the genus *Photorhabdus* are very potent producers of secondary metabolites, with biological activity. Many studies have reported the nematicidal activity of these bacterial metabolites. In this study, we evaluated the nematicidal activity of the entomopathogenic bacterium, *Photorhabdus luminescens* subsp. *sonorensis*, which represents a novel source of bioactive molecules with potential applications in agriculture. Bioactivity of metabolites produced by this bacterium was evaluated on the root- knot nematode, *Meloidogyne incognita;* and the *Poa annua* stem gall nematode, *Anguina pacificae*. Results from *in vitro* and *in planta* bioassays showed that *Photorhabdus l. sonorensis*' secondary metabolites have potent antagonistic activity, achieving complete mortality in vitro. Moreover, *in planta* assays showed reduction on gall formation for both plant-parasites. The extracts'composition was analyzed by TLC, HPLC-UV, and HPLC-MS. Results of this study are herein presented and discussed.

Contributed papers. Tuesday, 09:00. **74**

Potential of native nematodes as pest snail control agents in Australia

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A series of bioassays were conducted on four pest snails in Australia, *Cernuella virgata*, *Theba pisana*, *Cochicella barbara* and *Cochicella acuta*, to evaluate the efficacy of a native nematode (*Rhabditis myriophila*) as a residual biopesticide treatment. Nematode solutions (without formulation) at the rates of 0.5 x 10^6 per m^2 and 2.37 x 10^6 per m^2 were sprayed in 10 m x 10 m plots in a lentil field in spring 2011. The same number (200) of round snails (*C.virgata* and *T.pisana*) and conical snails (*C.barbara* and *C.acuta*) were exposed to the plots in application and monitored for mortality over a period of 28 days. A similar protocol was applied in the second field trial except for the nematode density (1.9 x 10^6 per m^2) and the timing (autumn 2012). The first field trial demonstrated the efficiency of *R. myriophila* on conical snails. The resulting snail mortalities were statistically higher than that of the negative control treatment ($P < 0.001$ for high concentration group and $P < 0.05$ for low concentration group). However, the nematode treatment caused no obvious snail mortalities on round snails. In the second field trial, nematodes caused relatively low snail mortalities on both conical and round snails. These results suggest the possibilities for use of *R. myriophila* to control conical pest snails in spring in Australia.

SYMPOSIUM (Microbial Control)
Tuesday, 10:30-11:30

Duking it out – interactions between the introduced microbial pest control agents and indigenous microflora

Symposium. Tuesday, 10:30. **76**

Insect Pathogen-Indigenous Microbe Interactions in the Rhizosphere

Cindy Fuller and Stefan Jaronski
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While it is known that insect pathogenic fungi inhabit soil, recent observations indicate that insect pathogenic *Metarhizium* species are also rhizosphere competent. *Metarhizium* has been found in natural association with roots of various plant species, and has been demonstrated to colonize the rhizosphere when introduced. Species-specificity has been observed between *Metarhizium* and the plants with which they are associated. Interactions of *Metarhizium* with insect cuticles and plants are dependent on adhesion proteins MAD1 and MAD2. Other factors influencing rhizosphere colonization have not been thoroughly investigated, including the effects of the rhizosphere microbiome. Furthermore, the lack of prediction of efficacy of insect pathogenic fungi in field tests might be explained by antagonism of rhizosphere flora. We used sugar beet (*Beta vulgaris* L.) as the host plant because of an ongoing project to develop *M. anisopliae* s.l. for use against the sugar beet root maggot, *Tetanops myopaeformis* (Röder) (Diptera: Ulidiidae). Sugar beets are colonized by numerous bacteria, many of which have antagonistic potential against plant pathogenic fungi including *Aphanomyces*, *Pythium* and *Rhizoctonia*. Our research extends the current knowledge to demonstrate that bacteria inhabiting the rhizosphere of sugar beets also have an antagonistic effect on conidial germination and vegetative growth of three strains each of the insect pathogenic fungi *B. bassiana* and *M. anisopliae* s.l. in an *in vitro* inhibition assay. Furthermore, these fungi differentially affected growth of various bacteria, suggesting that introduction of these fungi as biocontrol agents may alter the organization of the natural rhizosphere microbiome.

Symposium. Tuesday, 11:00. **77**

Determining the fate of introduced *Beauveria bassiana* GHA in agricultural fields and its impact on conspecific indigenous populations

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Beauveria bassiana commercial strains are widely used as mycoinsecticides for control of several insect pests, providing a biocontrol alternative to chemical insecticides. A key advantage of this fungus, and of other microbial control agents, is its potential to replicate and persist in the environment, offering continued suppression of insect pest populations. Exploiting this advantage, however, is commensurate with the need to determine the impact of mass releases of *B. bassiana* on non-target organisms. Risk assessment studies typically focus on beneficial organisms that may serve as alternate hosts of the pathogen. Little is known of the impact of mass-released fungal entomopathogens on conspecific indigenous populations in agricultural fields and on the likelihood of genetic recombination between introduced and indigenous strains of *B. bassiana.* A collaborative study on the fate of the commercially available *B. bassiana* strain GHA in agricultural fields in Maine and New York revealed field persistence of this strain at least 4 years following the last application and the displacement of indigenous conspecific strains following repeated applications. This displacement, however, appears to be temporary with the recovery of indigenous strains over time since the last application. Investigations on the likelihood of genetic recombination via the parasexual cycle showed that GHA is vegetatively incompatible with the more common indigenous strains. Furthermore, co-inoculations of Colorado potato beetle larvae with complementary *nit* mutants resulted in heterokaryon formation only between strains of the same vegetative compatibility group, suggesting that the self/non-self recognition system of the parasexual process is an effective barrier preventing genetic exchange between dissimilar strains in the field.

CONTRIBUTED PAPERS
Tuesday, 10:30-12:30

Viruses 1

Contributed paper. Tuesday, 10:30. **78**

Managing hytrosavirus infections in *Glossina pallidipes* colonies: Feeding regime affects the prevalence of salivary gland hypertrophy syndrome

Adly M. M. Abd-Alla[a*#], Henry M. Kariithi[a, b#], Abdul Hasim Mohamed[a], Edgardo Lapiz[a], Andrew G. Parker[a], and Marc J.B. Vreysen[a]
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Many species of tsetse flies are infected by a virus that causes salivary gland hypertrophy (SGH) syndrome and the virus isolated from *Glossina pallidipes* (GpSGHV) has recently been sequenced. Flies with SGH have a reduced fecundity and fertility. Due to the deleterious impact of SGHV on *G. pallidipes* colonies, several approaches were investigated to develop a virus management strategy. Horizontal virus transmission is the major cause of the high prevalence of the GpSGHV in tsetse colonies. Implementation of a "clean feeding" regime (fresh blood offered to each set of flies so that there is only one feed per membrane), instead of the regular feeding regime (several successive feeds per membrane), was among the proposed approaches to reduce GpSGHV infections. However, due to the absence of disposable feeding equipment (feeding trays and silicone membranes), the implementation of a clean feeding approach remains economically difficult. We developed a new clean feeding approach applicable to large-scale tsetse production facilities using existing resources. The results indicate that implementing this approach is feasible and leads to a significant reduction in virus load from 10^9 virus copies in regular colonies to an average of $10^{2.5}$ and eliminates the SGH syndrome from clean feeding colonies by28 months post implementation of this approach. The clean feeding approach also reduced the virus load from an average of 10^8 virus copy numbers to an average of 10^3 virus copies and SGH prevalence of 10% to 4% in flies fed after the clean fed colony. Taken together, these data indicate that the clean feeding approach is applicable in large-scale *G. pallidipes* production facilities and eliminates the deleterious effects of the virus and the SGH syndrome in these colonies. The combination between clean feeding system and antiviral drug valacyclovir with 300 µg/ml resulted in elimination of SGH from treated tsetse colony after six months of starting the treatment.

Contributed paper. Tuesday, 10:45. **79**

Microplitis demolitor* bracovirus (MdBV) persists in a semi-permissive host *Trichoplusia ni

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Polydnaviruses (PDV's) are DNA viruses associated with parasitoid wasps. The close association between PDV-carrying wasps and their hosts makes them an excellent model for studying host-pathogen interactions. *Microplitis demolitor* carries *M. demolitor* bracovirus (MdBV) and parasitizes the permissive host *Pseudoplusia includens*. In contrast, the related moth *Trichoplusia ni* is a semipermissive host for *M. demolitor*. Many of the factors responsible for the permissiveness of *P. includens* are well studied, but why *T. ni* is semipermissive remains unclear. Here we report that *T. ni* exhibited developmental resistance toward *M. demolitor* with wasp progeny developing successfully in younger hosts more frequently than in older hosts. Analysis of MdBV indicated that viral DNAs persisted in both early and late instar *T. ni* larvae and that virulence genes responsible for suppression of host immune defenses are constitutively expressed. However, members of protein tyrosine phosphatase (PTP) gene family were either not expressed or were only transiently expressed in *T. ni.* Taken together, these data identify the absence of PTP gene products as a potential factor involved in the developmental resistance of *T. ni* toward *M. demolitor*.

Contributed paper. Tuesday, 11:00. **80**

A Survey of Single-stranded RNA Viruses in the Tarnished Plant Bug, *Lygus lineolaris*

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Full length genome of a single-stranded RNA virus infecting the tarnished plant bugs (TPB) was previously characterized and published. Partial genomes of two additional RNA viruses were also characterized from field collected tarnished plant bugs by assembly of cDNA sequences obtained by high throughput sequencing. Characteristics of the viral genomes identified in TPB are being compared with known single stranded RNA viruses of other insects.

Contributed paper. Tuesday,11:15. **81**

***Wolbachia* increases host susceptibility to *Spodoptera exempta* nucleopolyhedrovirus**

Robert I. Graham[1], David Grzywacz[2], Wilfred L. Mushobozi[3], Kenneth Wilson[1]
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Wolbachia are common vertically transmitted endosymbiotic bacteria found in < 70% of insect species. They have generated considerable interest due to the capacity of some strains to protect their insect hosts against virus infections, and the potential for this to reduce vector competence of a range of human diseases, including dengue. In our study, we looked at the interactions of *Wolbachia* and a nucleopolyhedrovirus (SpexNPV) in the lepidopteran crop pest, African armyworm (*Spodoptera exempta*). We show that the prevalence and intensity of SpexNPV infection is positively associated with infection of *Wolbachia*. We also undertook laboratory bioassays to demonstrate that *Wolbachia* infection increases viral mortality by up to14 times. These findings suggest that rather than protecting their lepidopteran host from viral infection, *Wolbachia* instead make them more susceptible to this particular natural disease. This finding potentially has implications for the biological control of other insect crop pests.

Contributed paper. Tuesday,11:30. **82 STU**

Possible correlation between genetic diversity and viral activity in different Polish LdMNPV isolates.

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Crop and forests protection chemicals, often used in excess, cause irreversible damage in natural environment and can lead to harmful effects on humans and animals. Thus, biopesticides based on baculoviruses can be considered as an attractive alternative. Over 600 species vulnerable to baculoviral infection have been described till now. The great majority are larvaes of moths and butterflies (Lepidoptera). Many differences in pathogenicity of various strains of Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV) have been reported since 1970s. LdMNPV infects the gypsy moth, a pest which destroys deciduous forests and shrubs thus bringing huge economical loses. Viruses from diverse polish regions differ in activity (determined by the lethal dose level – LD and amount of inclusion bodies per larvae - OB) against their host, but the genetic reasons of this phenomena have not been investigated. During our experiments we determined and analyzed few (e.g. lef-8, lef-9, *egt*) genes of LdMNPV from different geographic regions, their nucleotide sequence and protein structure. These data were compared with levels of activity against their hosts and we have found possible correlation between mutations in DNA sequence and pathogenicity of baculovirus strain. Such information can help in future to screen for most virulent baculovirus strain without need for expensive and time-consuming procedures.

Contributed paper. Tuesday, 11:45. **83 STU**

A novel pea aphid antiviral defense strategy

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Aphids are major pests of important agricultural crops worldwide. Viral pathogens could have potential for use in the management of aphids. We aimed to identify novel aphid viruses and investigate aphid-virus interactions at the molecular level. A new aphid virus, Acyrthosiphon pisum virus-2 (APV-2) was assembled from small RNA sequencing data from the model aphid species, the pea aphid (*Acyrthosiphon pisum*). APV-2 is a positive sense, single stranded RNA virus in the family Dicistroviridae. The virus has a genome length of 9,888nt and encodes 3 major coat proteins. Analysis of virus-derived small RNA (vsRNA) from AP2 in pea aphid revealed an unusual profile of vsRNA reads ranging from 13 to 35nt. This is in contrast to the typical profile with a 22nt vsRNA peak from a second novel aphid virus, Aphis glycines virus (AGV) from the soybean aphid. The unusual vsRNA read profile from AP2 virus has striking similarities to that of the small RNA profile from *Buchnera aphidicola*, an obligate intracellular bacterium found in most aphid species. In addition, AP2 appears to localize to the gut and bacteriocytes of the pea aphid. Taken together, we hypothesize that RNases of symbiotic bacteria function in antiviral immunity against AP2 in the pea aphid. The degradation of viral RNA by host endosymbiotic bacteria represents a novel antiviral defense pathway.

Contributed paper. Tuesday, 12:00. **84**

Isolation and characterization of host ranges extended baculoviruses through co-infection approach in insect cells

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Host range of baculovirus is narrow and usually limits its applications. Here, we tried to extend host ranges of *Autographa californica* multiple nucleopolyhedrovirus (*AcMNPV*) and *Maruca vitrata* multiple nucleopolyhedrovirus (*MaviMNPV*) by co-infection approach and through a fluorescence-based selection method. The isolated host range extended baculoviruses was tentatively named A-M bac. A-M bac could finish the viral life cycle among *Spodoptera frugiperda* 21(*Sf*21) or High-Five (Hi-5) cells which are permissive for *AcMNPV*, and NTU-MV532 cells which is permissive for *MaviMNPV*. Interesting, infection of these cell lines with A-M bac virus produced high level of exogenous EGFP expression, especially in *Sf21* and Hi-5 cells, than EGFP-expressing *AcMNPV*. Furthermore, A-M bac could infect the *Spodoptera exigua* larvae, which can be infected by AcMNPV only through injection, through aerosol approach and the infection rate was about 20 %. Thus, A-M bac may be used for recombinant proteins production in the future. To identify the genes that might mediated the host range extension of A-M bac, the whole genome sequence of A-M bac was conducted. We found that a region nearby the polyhedron locus of *AcMNPV* was replaced by a 10.2-kb DNA fragment of *MaviMNPV* and resulted in lost of four ORFs (Ac-ORF603, Ac-bro, Ac-ctx, Ac-Orf12) and one homologous region (Hr1a). Furthermore, we also found some of these conserved ORFs showed relatively lower identities between *AcMNPV* and *MaviMNPV*, such as AcOrf4 or Ac-pe38. In conclusion, this hybrid virus might help to identify the host range factors and to elucidate mechanisms of host restriction among various baculoviruses to their insect hosts in the future.

Contributed paper. Tuesday, 12:15. **85**

Characterization of baculoviruses from the Martignoni collection
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47 samples from the Martignoni baculovirus collection were characterized by PCR amplification of the *lef-8* gene. This led to the identification of sequences from viruses that either were not present in the database, or had been identified, but not further characterized. These included an NPV and a GV from *Pseudoletia (Mythimna) unipuncta*, and NPVs from *Coloradia pandora*, the oak and hemlock looper (probably *Lambdina fiscellaria.), Peridroma* sp., the pine butterfly (probably *Neophasia sp.), Hemileuca* sp. (probably *H. eglanterina*, *Orgyia vetusta*, and several *Choristoneura* sp. A phylogenetic tree was constructed relating these viruses to their closest relatives in the database.

CONTRIBUTED PAPERS
Tuesday, 10:30-12:00

Diseases of Beneficial Invertebrates 1

Contributed paper. Tuesday, 10:30. **86**

Dynamics of the presence of IAPV in CCD colonies
Chunsheng Hou[1], Hadassah Rivkin[1], Yossi Slabezki[2] and Nor Chejanovsky[1]
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Viral pathogens were associated with dramatic collapse of honey bee colonies reported in the last years around the world. In particular, the Israeli acute paralysis virus was related to the Colony collapse disorder (CCD). Following the initial discovery of CCD in the US around 2006 we identified a group of colonies in Israel matching the hallmarks of CCD. These colonies were maintained by careful treatment and some of them were able to recover and survive for a long period. The aim of this study was to characterize viral pathogens present in the colonies and to follow up the status of these pathogens in them. Qualitative and quantitative RT-PCR-based diagnostics pointed out at IAPV as the major viral pathogen present in the colonies. Periodic examination of the IAPV status indicated that the viral titers were decreasing through the seasons. However, monitoring the ability of IAPV to replicate by molecular methods and bioassays, respectively, indicated that its infectivity was maintained. Our study suggests that in CCD colonies rescued before total collapse IAPV maintains an active replicative form that, given favorable conditions, confers it the property of rapid amplification.

Contributed paper. Tuesday, 10:45. **87 STU**

Different strategies of *Paenibacillus larvae* to evade the immune response of honey bee larvae
Gillian Hertlein, Lena Poppinga, Eva Garcia-Gonzalez, Anne Fünfhaus, Kati Hedtke, Elke Genersch
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Honey bees are among the most important productive livestock due to their indispensable role as commercial pollinators of many agricultural crops and fruit. Therefore, maintaining honey bee health and mitigating honey bee diseases is crucial for human food production and security. The epizootic American Foulbrood, caused by *Paenibacillus larvae*, is a fatal bacterial disease of honey bee brood able to kill entire colonies which leads to considerable losses in global apiculture every year. We recently identified and characterized four genotypes of *P. larvae* (ERIC I-IV) which differ in virulence, i.e. death of the infected larvae and collapse of diseased colonies proceed at different rates. First results on the molecular pathogenesis of *P. larvae* infections revealed that ERIC I and ERIC II developed different strategies to kill infected larvae. It has been shown that honey bee larvae mount an immune response against *P. larvae* infection by up-regulating expression of several antimicrobial peptides (AMP). We now show that this up-regulation also differs between the *P. larvae* genotypes with the fast-killing genotype ERIC II triggering a weaker immune response than the slowly killing genotype ERIC I. We hypothesize that the ERIC II-specific S-layer protein helps the bacteria to evade the larval immune system. In addition, ERIC II exclusively expresses immune inhibitor A (InhA), a protein known to be involved in AMP-degradation. Knock-out mutants for InhA show significantly reduced mortality in exposure bioassays suggesting that InhA plays an important role during pathogenesis of *P. larvae* infections.

Contributed paper. Tuesday, 11:00. **88**

Survey of chalkbrood fungi infecting alfalfa leafcutting bees in U.S. alfalfa seed fields
James, Rosalind R., Klinger, Ellen, G., Pitts-Singer, Theresa L.
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Alfalfa leafcutting bees (*Megachile rotundata*) are used for pollinating alfalfa in the U.S. and Canada. This solitary bee is susceptible to a fungal disease of the larvae called chalkbrood which kills, on average, 8% of the larvae. Overwintering bee larvae were collected for two years across the alfalfa seed growing region of the U.S. and visually examined for disease. PCR diagnostic techniques were then used to identify larvae infected with *Ascosphaera*, and selective primers were used for each of the seven *Ascosphaera* species known to infect this bee, and *A. apis* (the most common chalkbrood pathogen of honey bees). In the first year, none of the 726 lived bees tested were positive for *Ascosphaera*, so only dead larvae were evaluated by PCR the second year. In total, 736 dead larvae were tested for infections. Of these, 61% were *Ascosphaera*-positive, and 95% of those were infected with *A. aggregata*; 20% were co-infected with two or more *Ascosphaera* spp., nearly all of which included *A. aggregata* as one of the pathogens. *Ascosphaera proliperda* was the second most common pathogen, but mainly occurred as co-infections with *A. aggregata*. No *A. apis* or *A. acerosa* infections occurred. We confirmed that *A. aggregata* is the predominant chalkbrood pathogen of alfalfa leafcutting bees, and sublethal infections by *Ascosphaera* spp. do not persist into the host overwintering stage. We hypothesize that *A. aggregata* infections increase the ability of other *Ascosphaera* to infect, leading to the large number of co-infections found.

Contributed paper. Tuesday, 11:15. **89 STU**

Pathogenicity of mixed infections of *Ascosphaera* in solitary and social bees
Klinger, Ellen G. [1, 2], Vojvodic, Svjetlana[3], and James, Rosalind R.[2]
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In multiple infections, the framework underlying expressed pathogen virulence to their hosts can be characterized as superinfective or co-infective. In superinfection, host mortality rates and pathogen reproductive propagule levels (e.g. spores) are dominated by the most virulent pathogen. In co-infection, host mortality and pathogen propagule production levels are intermediately controlled by each pathogen. Co-infection may be selected for over superinfection in several cases, one of which is high relatedness of the infecting pathogens. The *Ascosphaera* are a group of entomopathogens causing a larval disease known as chalkbrood in several groups of social and solitary bees. We inoculated the larvae of two bee species (*Apis mellifera* and *Megachile rotundata*) with spores of *Ascosphaera* species (one highly virulent species-specific pathogen, and one with low virulence), singly and

in combination. The species-specific *A. mellifera* pathogen was more closely related to the low virulence pathogen than was the species-specific *M. rotundata* pathogen. Based upon host mortality, the virulence of all mixed infections in both bees mirrored that of the most virulent pathogen only. However, when the number of spores produced post-infection were examined, less closely related pathogen infections in *M. rotundata* appeared to be co-infective, and highly related mixed infections in the honey bee remained superinfective. We conclude that *Ascosphaera* pathogen phylogenetic relationships are not a good predictor of the outcome of multiple infections, and inclusion of infective propaugle production is critical in accurate description of the pathogen virulence framework in these mixed infections.

Contributed paper. Tuesday, 11:30. **90**

Histopathology of infectious diseases of the honey bee (*Apis mellifera*)

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Various infectious diseases of the honey bee (*Apis mellifera*) are a threat to honey bee health, causing hive weakness and colony losses. In recent studies pathogens affecting honey bees (bacteria, viruses, and fungi) have been identified etiologically by various laboratory methods, like polymerase chain reaction (PCR) and bacteriological reference methods. Influences of the pathogens to honey bee tissues have not been systematically investigated so far. Aim of this work is 1) to establish standardized microscopic and histopathological methods for preparation and staining of honey bee tissue thin sections, 2) to illustrate normal healthy tissue structures of every life cycle of the honey bee and 3) to identify tissue alterations caused by different pathogens. To this aim, honey bees of different ages were infected in the laboratory or directly isolated of diseased colonies and subsequently analyzed. Tissue samples of both healthy and diseased honey bees as well as whole animals were fixed in formalin or ethanol at distinct time points post infection and paraffin sections were prepared thereof. Subsequently these sections were stained routinely with hematoxylin&eosin or Warthin&Starry and microscopically analyzed. Pathogen identification was performed by species-specific fluorescence *in situ*-hybridization (FISH) to identify infected cells and to understand the pathogens' life cycle during the infection process. This work is the first histologically approach to detect and illustrate various infectious diseases of the honey bee and will finally present a compilation of figures comparing diseased and healthy honey bee tissue structures.

Contributed paper. Tuesday, 11:45. **91**

Are pesticides affecting pathogen levels and immunity in honey bees?

Brenna E. Traver[1], Nels G. Johnson[2], Katelyn M. Catalfamo[3], Haley K. Feazel-Orr[3], Troy D. Anderson[1], and Richard D. Fell[1]
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Honey bee colony losses continue to be a problem for the beekeeping industry. Colony losses are most likely the result of multiple factors. Here we report the effects of pesticide treatments on pathogen levels and immunity factors in honey bees. Three different pesticides were used: chlorothalonil, a commonly used fungicide; fumagillin, an antibiotic used for *Nosema* control; and *tau*-fluvalinate, an acaricide used for varroa mite control. We collected samples of bees pre-treatment and 2 and 4 weeks post-treatment. Analysis for the fall showed that the three treatments had no significant effect on *N. ceranae* levels, nor did *N. ceranae* levels vary over time; however, there were significantly fewer *N. ceranae* infections 4 weeks post-treatment compared to 2 weeks post-treatment ($p < 0.01$). We examined phenoloxidase (POX) and glucose oxidase (GOX) as measures of individual and social immunity, respectively. POX activity did not vary across treatments but overall POX activity was significantly higher at two weeks post-treatment compared with pre-treatment activity ($p < 0.01$) but was not different from levels at 4 weeks post-treatment. GOX activity was not affected by any treatment or treatment timing. Furthermore, the correlation between GOX and POX was not affected by treatment or the treatment timing. Our results suggest that exposure to chlorothalonil, *tau*-fluvalinate, and fumagillin in the fall do not negatively affect colonies. Our study is continuing with treatments in spring and summer of 2013 to monitor possible changes over the season when honey bees face different stresses.

WORKSHOP (Fungi and Microbial Control)
Tuesday, 11:30-13:00

What's the name of my fungus?

Workshop. Tuesday, 11:30. **92**

Current status of phylogenetic reclassifications: Here today and gone tomorrow

Richard A. Humber
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The recent phylogenetic reclassifications of *Beauveria* and *Metarhizium* based on analyses of multiple genes and complemented by morphological documentations of the taxa have substantially increased the number of species recognized in these genera, although the analyses and publication of the results involving several taxa within *Metarhizium* (primarily the *M. flavoviride* species complex) still remain to be completed. Nothing about these studies should be considered now to be ultimately definitive since isolates of these genera from so many more parts of the world remain essentially unstudied. Further refinements in the molecular approaches used for studying these fungi may also further split some of the existing large and globally distributed groups. For example, the *B. bassiana* and *M. anisopliae* clades in their current taxonomically restricted senses remain extremely large and comprise much inherent genetic variability. It is also worrisome that just at a time when phylogenetic revisions of these genera have brought a new sense of order and predictability to them that the 2011 changes in the rules of nomenclature applicable to pleomorphic fungi will forcibly impose chaotic expansions of generic concepts on these hypocrealean fungal entomopathogens that will, in many senses, erase these recent advances.

Workshop. Tuesday, 12:00. **93**

Field to Phylogeny: Molecular identification of *Beauveria* and *Metarhizium* species

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Accurate identification of fungal biocontrol agents from species to individual strains is critical to their implementation in insect control programs. The precision of species identification has improved greatly with the advent of molecular methods. Multigene phylogenetic analyses of the biocontrol agents *Beauveria bassiana* and *Metarhizium anisopliae* have revealed species concepts defined by morphology actually represent complexes of cryptic species. These multigene analyses have relied on loci commonly used in fungal systematics and likely underestimate diversity within these complexes. By leveraging the available genome resources for *Beauveria* and *Metarhizium* we have developed highly variable genus specific molecular markers from intergenic regions between conserved protein coding genes. Analyses of phylogenetic informativeness have shown these markers have greater signal than those traditionally used in fungal phylogenetics, and are able to diagnose diversity at and below the species level. Species identification by sequencing is now a straight forward process when incorporated into the existing phylogenetic framework. The most informative locus for *Beauveria* (BLOC) and *Metarhizium* (Mz_IGS3)

are suitable for rapid species identification of unknown isolates to species in most cases. The application of these markers has revealed multiple phylogenetic partitions within currently recognized species and emergent phylogeographic structure of species distributions. In this talk we will present methodologies to incorporate unidentified isolates into the working phylogenetic framework. Given the potential to resolve diversity at such a fine scale, we recommend prudence and discretion in the naming of new taxa based on the use of these markers until phylogeographic trends and diversity come into focus.

Workshop. Tuesday, 12:30. **94**

Molecular tools for strain detection and population genetic analyses of *Metarhizium* and *Beauveria* spp.
Jürg Enkerli, Andy Lutz, Franco Widmer
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Population genetic analyses can provide many insights into the underlying biology of a fungus, including its origin, adaptation processes, and mating/reproductive system. They also provide information required when investigating the success of applied Biocontrol Agents. Assessment of the within species genetic diversity represents a central component of such analyses and various approaches have been applied for such purposes, they include universally primed PCR, repetitive element PCR, single nucleotide polymorphism, simple sequence repeats (SSR), amplified fragment length polymorphism or sequence analyses. SSR marker are particularly valuable in this context because they allow differentiation of individuals with high resolution and are amenable to automated high-throughput analyses. For the two entomopathogenic genera *Metarhizium* and *Beauveria* SSR markers have been isolated for *M. anisopliae s.l.* (Enkerli et al 2005, Oulevey et al. 2009), *B. bassiana s.l.* (Rehner et al. 2003, Meyling et al. 2009) and *B. brongniartii* (Enkerli et al. 2001). However, recent taxonomic revisions within the two genera resulted in ambiguities as to which SSR markers can be applied to which of the species. We have investigated the transferability of the 41 *Metarhizium* SSR markers available to resolve the ambiguity. The study revealed that the transferability strongly varies among *Metarhizium* spp. For instance 28 and 18 markers are applicable to *M. brunneum* and *M. robertsii*, respectively. Results have allowed definition of specific maker sets for efficient population genetic analyses of single *Metarhizium* spp. Similar investigations will have to be performed for *Beauveria* SSR markers to provide genotyping tools with appropriate specificity.

WEDNESDAY - 14 August

SYMPOSIUM (Fungi)
Wednesday, 08:30–10:30

Forty years of ARSEF: success of an essential germplasm resource

Symposium. Wednesday, 08:30. **95**

Spreading culture: From a refrigerator to the whole world
Richard A. Humber
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Once upon a time Richard Soper had a small collection of about 100 tubes of mostly entomophthoralean fungal cultures in his USDA-ARS lab at the University of Maine when I began my postdoc. Don Roberts' joined the lab in 1977 on sabbatical and forged an unbreakable link that brought these fungi and us to the new Boyce Thompson Institute facilities at Cornell and set a course for the rapid expansion of both the size and scope of this collection. With Don Roberts as its largest contributor, the collection has grown steadily into the formally organized, general service culture collection with ca 12000 isolates and the internationally collaborative resource that it is today. While ARSEF's methodologies continue evolving and improving, the goal of providing reliable and stable storage facilities, ready access to cultures, and a maximum level of service to a large, global clientele remains steadfast. ARSEF and its clients–depositors and recipients–have benefitted from such goals and operational approaches. In addition to its germplasm services, ARSEF also serves as a major informational resource through its extensively indexed catalogs, by providing diverse training to students and professional scientists on site in Ithaca and throughout the world, and as a center for and collaborator in much significant research on the systematics, taxonomy, and organismal biology of fungal entomopathogens. None of this could have been possible without continued federal funding through the USDA-ARS and the partnership of the ARSEF clientele with whom the collection proudly shares its reputation, its credibility, and its accomplishments.

Symposium. Wednesday, 08:50. **96**

Importance of exploration to the success of ARSEF, an essential germplasm resource for entomopathogenic-fungi research
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Diversity is a required trait of a truly useful collection of fungi, and this trait is well represented in the holdings of ARSEF. For entomopathogenic (EP) fungi, diversity includes a wide range of a.) fungal EP species; b.) arthropod hosts; and c.) geographic regions. The worldwide exploration necessary to build ARSEF has involved numerous scientists, and I am pleased to be participant. My career path has included initiating several projects that involved seeking and culturing several thousands of new isolates of EP fungi from a wide range of international and USA sites. For a number of years, I maintained a personal culture collection of several hundreds of isolates. Richard Soper and Richard Humber's USDA/ARS unit was housed within my parent institution, Boyce Thompson Institute for Plant Research, for ten years starting in 1978; and during this time it became clear that the most efficient and safest archiving of my cultures would be to deposit them in ARSEF. In years since, ARSEF has served this function for several international surveys for EP fungi. This is truly an invaluable service to both the contributing scientists and to Science. My lab currently is seeking cultures of fungal pathogens of grasshoppers and Mormon crickets in >30,000 USA soil samples provided by collaborators working in the natural habitats of these pest insects. Another valuable source has been donations of isolates to us while visiting overseas scientists, which we deposited in ARSEF with credit to the contributor. In addition to insect pathology researchers, users of ARSEF include academicians, government scientists and occasional commercial organizations. The ARSEF collection is unique and of crucial importance to many scientific endeavors worldwide; and all scientists interested in entomopathogenic fungi should join forces to assure its continuance. **Key words**: Entomopathogenic fungi; culture collection; germplasm conservation.

Symposium. Wednesday, 09:10. **97**

Genome-driven insights into the phylogeny, population biology and molecular ecology of *Beauveria* and *Metarhizium*
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Beauveria and *Metarhizium*, like most fungi, live largely hidden lives. Their small size, simple morphology and low resolution with first generation molecular tools impeded past efforts to clarify their life histories, their roles in regulating insect populations and the adaptive forces that shape their evolution. However, recently available genome sequences for these taxa has enabled the development of new molecular tools better suited for reconstructing their phylogenetic histories, delineating species boundaries, developing accurate species identification methods, and for determining their population genetic structure. In conjunction with the extensive

germplasm resources contained within public culture banks such as the ARS Collection of Entomopathogenic Fungi (ARSEF), we are using these tools to rapidly clarify the most intimate details of their evolutionary histories and to develop novel insights into their species diversity, phylogeography and population biology. In this talk we review the taxonomic limits and phylogenetic structure of *Beauveria* and *Metarhizium* as developed from recent multilocus phylogenetic analyses. In addition, we will introduce the remarkable phylogenetic and phylogeographic diversity within the *B. bassiana* and *M. anisopliae* species complexes made possible with newly developed molecular tools via comparative genomics approaches. The greater precision in delineating species boundaries with these new tools now make possible incisive population studies that can will address the many persisting questions about the reproductive systems, population biology, phylogeography and epidemiology of these ubiquitous and ecologically important organisms.

Symposium. Wednesday, 09:30. **98**

Studies on host-pathogen interactions using isolates from the ARSEF collection.

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My laboratory has published more than 100 papers using isolates from the ARSEF collection. Most of the 300 plus ARSEF isolates we investigated were *Metarhizium* spp, but we have also studied *Beauveria, Nomuraea, Verticillium, Paecilomyces, Erynia, Fusarium* and *Conidiobolus* spp. Our studies have employed these fungi as models for understanding how pathogens respond to changing environments, initiate host invasion, colonize tissues, and counter host immune responses. We have also addressed the mechanisms by which new pathogens emerge with different host ranges as well as fungal behavior and evolution, fungal toxins, molecular biology of fungi, and insect control with fungi. Recent work in our lab employing ARSEF strains will be reviewed.

Symposium. Wednesday, 09:50. **99**

How does the ARSEF collection contribute to studies on ecology of insect pathogenic fungi?

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In order to study ecology of insect pathogenic fungi, a reference collection is essential. USDA collection has for decades served as one such reference. In articles, where scientists have published about natural occurrence of these fungi and include comparison with well characterized isolates, we may often find one or more ARSEF isolates in the tables and figures. Concerning the Entomophthoramycota the ARSEF has had significance, which cannot be overestimated. As the sole collection able to maintain safely a high number of isolates from the different genera of Entomophthoramycota in liquid cultures (including fastidious species growing as protoplasts), the significance of ARSEF and its curator Richard Humber cannot be overestimated. I will provide some examples of the specific importance ARSEF has had for studies on ecology of Entomophthoramycota.

Symposium. Wednesday, 10:10. **100**

The ARSEF collection and 40 years of microbial biocontrol with entomopathogenic fungi

Stephen P. Wraight
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The past four decades have seen a dramatic increase in our capacity to manipulate entomopathogenic fungi as pest control agents. Advances in mass production, formulation, packaging, and application technologies combined with development of improved strategies for deployment of these control agents, especially as components of integrated pest management programs, have greatly stimulated worldwide development efforts. A survey of fungus-based biopesticides published in 2007 identified > 170 products, and many new products have been developed in the last five years. Even though markets for these products have often proven difficult to establish and slow to expand in the face of competition from chemical insecticides, development is persistently driven by public demands for safer pesticides and sustainable pest control systems. The USDA-ARS Collection of Entomopathogenic Fungi (ARSEF), officially registered with the World Data Center for Microorganisms in 1985 has played a major role in support of this development, with acquisitions of fungal pathogens increasing steadily from ca. 2,000 isolates in 1985 to >12,000 today. A number of currently marketed fungal strains comprising diverse mycoinsecticide products trace their origin to the ARSEF collection, including the widely marketed strain GHA of *Beauveria bassiana*, registered and commercialized in 1995. Innumerable fungal isolates from the ARSEF collection have been screened for their microbial control potential and used as subjects in the above-mentioned biopesticide research and development efforts. This presentation will highlight key developments in fungal microbial control that have involved pathogens with ties to the ARSEF collection.

SYMPOSIUM (Microsporidia)
Wednesday, 08:30–10:30

Graduate student studies of microsporidia and other protists

Symposium. Wednesday, 08:30. **101 STU**

Genetic architecture underlying variation in *Caenorhabditis elegans* host resistance to natural microsporidia infection

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Animals are embedded in complex co-evolutionary relationships with pathogens, and the specific genetic consequences associated with most of these interactions remain undefined. Microsporidia are fungal-related obligate intracellular pathogens of all animal phyla, yet the genetic architectures underlying host resistance mechanisms are unknown. We demonstrate natural variation among strains of *Caenorhabditis elegans* in their ability to resist *Nematocida parisii*, a microsporidian species isolated from wild-caught nematodes. Resistance varies post-invasion, mediating the growth of the pathogen and host survival. We utilize quantitative genetic analyses to map the genetic architecture of resistance to at least five quantitative trait loci. Two of these loci are confirmed contributors to resistance or susceptibility to *N. parisii*. These results exhibit the complex genetic nature of resistance mechanisms that have evolved under a ubiquitous host-pathogen interaction, and pave the way for establishing the molecular bases of these interactions.

Symposium. Wednesday, 08:50. **102 STU**

Microsporidia and the two-spotted lady beetle *Adalia bipunctata* L.

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The two-spotted lady beetle, *Adalia bipunctata* L., is an important natural enemy that is used for biological pest control in North America and Europe. *A. bipunctata* are known to host a wide variety of symbionts, including microsporidia. Microsporidia are common pathogens of other lady beetle species and often cause chronic, debilitating disease by reducing host fitness. Two species of microsporidia infect *A. bipunctata*. *Nosema coccinellidae* infects field-collected beetles in Poland and *Tublinosema hippodamiae* has been transmitted from the convergent lady beetle, *Hippodamia convergens*,

to *A. bipunctata* under laboratory conditions. However, a third, undescribed microsporidium was recently isolated from field-collected *A. bipunctata* in Nova Scotia, Canada. The objective of this presentation is to provide a review of the microsporidian pathogens that infect *A. bipunctata*, including a formal description of the undescribed pathogen from field-collected *A. bipunctata* by means of pathogen ultrastructure, tissue pathology and molecular characterization. The host specificity and effects of multiple microsporidian infections within *A. bipunctata* will also be described.

Symposium. Wednesday, 09:10. **103 STU**

Immune response of *Lymantria dispar* to naturally occurring intracellular pathogens

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Although insects lack the adaptive immune system of vertebrates, they do possess a complex innate defense system. Recognition of foreign microbes leads to a series of defense actions including signal proteins and antimicrobial peptides, as well proteins involved in the phenoloxidase cascade that defend against invading pathogens. Although ecologically and physiologically relevant routes of infection are primarily oral ingestion or entry through the tracheal system or cuticle, models of the humoral immune response in insects are typically produced by injection of facultative bacteria or foreign objects into the host. Immune responses are key factors determining the level of susceptibility of hosts to their natural enemies but comparative studies are rare, primarily because hosts or their natural pathogens are not easily available or produced. We chose the European gypsy moth, *Lymantria dispar* (L.) as our model species because its natural microbial enemies are well studied and available. We used a proteomic approach to study the induction of the immune response of gypsy moth larvae inoculated via oral ingestion to naturally occurring pathogens including the microsporidia *Vairimorpha disparis* and *Endoreticulatus schubergi*, nuclear polyhedrosis virus (*Ld*MNPV), cytoplasmic polyhedrosis virus, and the microbial pesticide *Bacillus thuringiensis* kurstaki. Fourth instar larvae were inoculated and hemolymph was collected at 8, 18, and 36 hours post inoculation. Protein expression and abundance were quantified using 2-D gel electrophoresis with computer-assisted analysis of 2-DE patterns. We compared protein expression among treatments to determine if different pathogens with different host tissue targets induce differential expression of immune proteins.

Symposium. Wednesday, 9:30. **104 STU**

The potential use of a host specific biological treatment against locusts

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Large swarms of locusts have the potential to destroy 100.000 tons of crops a day in Africa. Especially the desert locust (*Schistocerca gregaria*) which recently (March, 2013) caused serious problems in the Middle East.They can spread over an area of 32 million km² and have an impact on 1/10 of the world population. In Africa, the yearly costs for prevention is USD 3.5M and the agro-economical costs of the last big swarm (2003-2005) were estimated at USD 2.5B. The prevention comprises field monitoring and the use of pesticides. Mainly organophosphates are used for the control of locusts, however more and more of those chemicals are banned due to the toxicity to the environment. It is also a very time consuming process and not all areas can be reached. Biological control agents provide an alternative such as the use of fungal spores which are commercialized as green muscle®. However, this works slowly and requires continuous spraying. Moreover, some areas in Africa are not accessible for spraying strategies. Here, we propose the use of an eugregarine (*Gregarina garnhami*) as a Trojan horse for locust control. *G. garnhami* is a natural parasite of *S. gregaria*, but causes no harm to his host. We want to investigate how we can load this parasite with an armory of choice (e.g. peptide toxin) to tackle locust swarming. For this, we have initiated a genome sequence survey of *G. garnhami* that will be used as a starting point for genetic engineering of *G. garnhami*.

Symposium. Wednesday, 9:50. **105 STU**

Studying the molecular and cellular evolution of intranuclear microsporidia in crabs

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Enterospora canceri is an intranuclear parasite recently isolated from European edible crabs. This parasite has a close phylogenetic affinity with *Enterocytozoon bieneusi*, a parasite responsible for most microsporidiosis infections in HIV patients. Microsporidian infections in humans are associated with diarrohea, wasting and keratitis. The close phylogenetic relationship between *E. canceri* and *E. bieneusi* a crustacean and mammalian parasite respectively is surprising. Apart from its infective spore stage, *E. canceri* lives its entire life cycle within host nuclei. *E. bieneusi* is also seen in close proximity to the host nuclei but never entirely within it. This strong affiliation with host nuclei in both species may highlight the presence of a novel host nuclei-dependent metabolic process but most importantly, it raises the question of how the crab parasite obtains energy from within its host nuclei; an environment completely secluded from host mitochondria (the source of energy for most microsporidia). Interestingly, *E. bieneusi* has lost all glycolytic pathways and is therefore apparently entirely reliant on its host to fulfill its energy requirements. A recently discovered crab parasite, *Hepatospora* sp. forms a phylogenetic sister group to both *Enterospora and Enterocytozoon* spp. and has no association with the host nucleus. My work aims to compare the genomes of these three parasites to identify the energy-harnessing/transporter proteins they employ that are specific to their host environment. It will also tell us whether loss of glycolytic pathways is unique to *E. bieneusi* and if so, when in evolutionary history this occurred.

CONTRIBUTED PAPERS
Wednesday, 08:30-10:30

Viruses 2

Contributed paper. Wednesday, 08:30. **106**

A potential role for effector caspases CASPS18 and CASPS19 in midgut escape of Sindbis virus in *Aedes aegypti*

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The midgut epithelium is the first target of arboviruses when they invade the arthropod vector. To establish a disseminated infection, arboviruses must cross the midgut basal lamina (BL), an extracellular layer that is secreted by epithelial cells and prevents passive diffusion by viruses. We are using Sindbis virus (SINV) and the mosquito vector *Aedes aegypti* to understand how arboviruses escape from midgut and establish systemic infections. During baculovirus infection in lepidopteran larvae, midgut infection initiates a cascade of protease activation in which matrix metalloproteases (MMPs) activate effector caspases, leading to cleavage of BL proteins and remodeling of the BL lining tracheal cells associated with the midgut, which allows baculovirus to escape the midgut. We hypothesize that the MMP-caspase-BL remodeling pathway is also used by arboviruses to escape the midgut. Prime candidates for caspase involvement in midgut BL remodeling are CASPS18 and CASPS19, effector caspases related to *Drosophila* Decay. Although CASPS18 itself does not have enzymatic activity, it has been shown to act as a decoy caspase that is able to enhance

the activity of CASPS19. Although the levels of CASPS18 and 19 transcripts and proteins in midgut were not altered by SINV infection, CASPS19 was cleaved in midgut following a blood meal. Immunofluorescence using antisera specific for CASPS18 and 19 revealed that CASPS18 and 19 were expressed in tracheal cells associated with midgut. SINV was also found in tracheal cells in SINV-infected midguts, suggesting that SINV may use the tracheal system to establish systematic infection.

Contributed paper. Wednesday, 08:45. **107**

Strong selective pressure against a recombinant Sindbis virus that induces apoptosis in the midgut of the mosquito *Aedes aegypti*

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Our laboratory recently reported that *Aedes aegypti* mosquitoes which were injected with dsRNA corresponding to the anti-apoptotic gene *Aeiap1* exhibited high levels of midgut apoptosis, and this resulted in enhanced replication and midgut dissemination of Sindbis virus (SINV) following an infectious blood meal. Similarly, silencing the initiator caspase *Aedronc* resulted in decreased SINV replication and dissemination. These results could be interpreted as indicating that apoptosis is required for efficient SINV replication and spread. However, the gene silencing approach affects both infected and uninfected cells, and could have secondary effects. As an alternative approach, we have utilized an alphavirus transducing system to construct a recombinant SINV that induces apoptosis. Oral infection of *A. aegypti* with a SINV expressing the pro-apoptotic *Drosophila* gene *reaper* (MRE/Rpr) induced apoptosis in infected midgut cells, while control viruses with similar size non-coding inserts did not. Replication of MRE/Rpr was reduced and delayed compared to control virus at early time points, but the titers of the two viruses were similar by 7 dpi. Sequencing of plaque-purified viruses obtained from mosquitoes infected with MRE/Rpr revealed that at 3 and 5 dpi, at least half of the mosquitoes assayed contained a mixture of viruses having either intact or deleted *reaper* inserts. However, all control viruses obtained from infected mosquitoes contained intact inserts, even up to 7 dpi. These results suggest that there was strong selective pressure in midgut against viruses expressing Reaper, indicating that if apoptosis is triggered in infected cells, it can reduce SINV infection in *A. aegypti*.

Contributed paper. Wednesday, 09:00. **108**

Inactivation of the budded virus of *Autographa californica* M nucleopolyhedrovirus by gloverin

Daniela A. Moreno-Habel[1], Ivan M. Biglang-awa[2], Paul M. M. Weers[2], and Eric J. Haas-Stapleton[1]
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Antimicrobial peptides are generated in insects exposed to pathogens for combating infection. Gloverin is a small cationic antibacterial protein whose expression is induced in the hemocytes and fat body cells of *Trichoplusia ni* larvae exposed to bacteria. The purpose of this study was to determine the role of gloverin during baculovirus infection. We found that gloverin expression is induced in *T. ni* systemically infected with the baculovirus *Autographa californica* M nucleopolyhedrovirus (*Ac*MNPV). Two gloverin genes were cloned from the hemocytes of *T. ni* larvae and expressed in *Sf*9 cells. The affinity purified gloverin proteins reduced the quantity of infectious *Ac*MNPV BV as measured *in vitro* by plaque assay using *Sf*9 cells. Nanomolar concentrations of affinity purified gloverin protein caused calcein to be rapidly released from unilamellar vesicles comprised of phosphatidylglycerol, but not from vesicles made up of phosphatidylcholine, suggesting that gloverin interaction with membranes is rapid and affected by membrane charge. Both the BV inactivation and calcein release activities increased with higher concentrations of gloverin. When *Sf*9 cells expressing GP64 were incubated in the presence of gloverin and the pH of the media reduced, the rate of membrane fusion was reduced relative to cells incubated with similar quantities of serum protein. These results demonstrate that gloverin is an antiviral protein that interacts with vesicle membranes to cause the contents to be released and affects GP64-mediated membrane fusion.

Contributed paper. Wednesday, 09:15. **109**

Genomic adaptation in the bracovirus of *Cotesia sesamiae* identified by targeted resequencing.

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Cotesia sesamiae are small parasitoid wasps parasitizing over twenty lepidopteran African stem borer species. It is thought that local adaptation to these different host species is mediated by their symbiotic bracoviruses (BVs). BVs derive from nudiviruses and have been stably integrated in the wasp genome. The wasp use BVs to introduce ~150 genes in parasitized caterpillars, inducing immuno-suppression and allowing wasp larval development. In *C. sesamiae*, different alleles of the CrV1 BV gene explain parasitic success in a particular host species. Nonetheless, other BV genes could be involved in wasp local adaptation or specialization to their lepidopteran hosts. To investigate this, we focused on the BV genome of 25 samples representative of different African *C. sesamiae* populations. As we worked with tiny organisms, we used custom-made targeted sequence capture to enrich our sequence library in BV genomes (257 kb) prior to Illumina resequencing. This proved to be a very efficient technique as we obtained high target coverage (1100X) and high percentage of mapped reads (90%) for all *C. sesamiae* populations and for the more distant outgroups. First, we used population genetics tools to identify regions under strong divergent selection by comparing nucleotide diversity (π) and genetic differentiation (FST) along the BV genome and between populations. Secondly, we used a phylogenomic approach to establish the evolutionary relationships between these populations using BV sequence of three outgroup species. Third, comparative genomics helped to assess the effects of particular mutations and identify sites evolving under positive selection. Last, we compared the molecular evolution of all orthologous BV genes both between and within populations and measured the rate of non-synonymous versus synonymous substitutions under a branch-site evolutionary model. Overall our results indicate that different BV genes are at play depending on local host context.

Contributed paper. Wednesday, 09:30. **110**

Functional comparison of the Group I and Group II alphabaculoviruses, *Autographa californica* multiple nucleopolyhedrovirus and *Mamestra configurata* nucleopolyhedrovirus, replication genes *DNA polymerase* and *lef1*

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In this study the functional conservation of function of the baculovirus replication genes *DNA polymerase* (*DNApol*) and *Lef1* of group I and group II alphabaculoviruses was investigated. *DNApol* and *lef1*of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) were substituted with the homologous genes from *Mamestra configurata* NPV-A (MacoNPV-A). The AcMNPV *dnapol* and *lef-1* knockout viruses were generated followed by repair with either the AcMNPV or MacoNPV-A wild-type genes and analyzed for virus replication. The knockout viruses were also rescued by

co-transfection with plasmids expressing chimeric constructs containing conserved regions of AcMNPV DNApol or LEF-1 substituted by homologous regions of the MacoNPV-A proteins. The aim was to investigate the functional significance and virus specificity of the whole protein and each domain. Analysis of time-courses of transfected cells of DNApol chimeric plasmids revealed that the AcMNPV DNApol exonuclease domain and polymerase regions-I and -II are not virus specific and can be substituted functionally by MacoNPV-A homologous regions. However, the AcMNPV polymerase regions-III, -IV and -V were found to be essential to rescue the AcMNPV *DNApol* knockout virus. Plaque assay and growth curve analysis of the LEF-1 chimeric constructs showed that MacoNPV-A LEF1 or chimeric constructs were unable to rescue the AcMNPV *lef1* knockout virus. These results indicate that AcMNPV *lef-1* and its domains are virus specific. The MacoNPV-A LEF1 and chimeric proteins were also analyzed for protein-protein interaction with AcMNPV LEF2 using a two-hybrid system. All constructs were found to bind LEF2 suggesting that loss of protein interaction was not responsible for loss of function.

Contributed paper. Wednesday, 09:45. **111 STU**

Baculovirus IE2 Forms Novel Visible Nuclear Cage-like Structure as Strong Transcriptional center

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Baculovirus expression system has been long known as a high efficient tool for eukaryotic protein production because of its ease of use and the versatility of its system. Baculovirus IE2 functions as a transcriptional regulator to *trans*-activate several viral genes in its virus life cycle. In this study, we discovered that IE2 can dramatically up-regulate CMVie and SV40 promoters both in VeroE6 and U-2OS cells. Unlike conventional activators, the activation is achieved by the formation of a novel visible nuclear cage-like structure (IE2 NC), where it recruits high concentration of G-actin and closely associates with highly condensed RNA polymerase II for the production of mRNA. RING and coiled-coil domains of IE2 are critical for the gene activation as well as the formation of the nuclear cage-like structure. Using real-time confocal analysis, we have shown the dynamic interaction between IE2 NC, nuclear actin and RNA polymerase II. We also have found that both nuclear actin and RNA polymerase II can continuously fill in IE2 NC after its formation, showing potential roles of actin in the transportation of transcription machinery into IE2 NC, which creates a center for tight association between IE2 and the host transcriptional machinery for strong activation of viral genes. Different from our knowledge that transcription is a minute structure only visible in fixed samples under electron microscopy and barely visible under light microscopy, our results provide a novel live transcriptional system visible under light microscopy for step-by-step studies of gene transcription.

Contributed paper. Wednesday, 10:00. **112**

Baculovirus photolyases and biological rhythm

Magdalena A. Biernat[1,2], Ines Chaves[2], Just M. Vlak[1], Gijsbertus T.J. van der Horst[2], Monique M. van Oers[1]
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Chrysodeixis chalcites nucleopolyhedrovirus encodes two photolyase-like proteins (PHR1 and PHR2), of which PHR2 has DNA repair activity. So far we have not been able to show the presence of these proteins in baculovirus particles, a prerequisite for the ability to repair UV-damaged DNA prior to infection; nor to restore the infectivity of UV-inactivated viral occlusion bodies by illumination with visible light, the normal way to activate photolyases. However, we have recently discovered that PHR2 can interact with components of the murine circadian clock and can functionally replace cryptochromes in imposing periodicity. Photolyases and cryptochromes belong to the same family of flavoproteins, but PHR2 is, together with the *Potorous tridactylus* PHR protein, unique in having both DNA repair and cryptochrome-like functions. Based on the information presented above the current hypothesis is that baculovirus PHR proteins do not have a primary function in protecting the viral genome from UV damage, but adjust the circadian clock in infected caterpillars, thereby interfering with the circadian rhythm of the insect larvae. Alterations in activity patterns over day and night may modulate virus yields and transmission rates. Besides inducing climbing behaviour and hyperactivity in infected larvae, adjusting circadian rhythm may form a novel way of host behavioural manipulation by baculoviruses.

Contributed paper. Wednesday, 10:15. **113**

The baculovirus core gene *ac83* is required for nucleocapsid assembly and *per os* infectivity of *Autographa californica nucleopolyhedrovirus*

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Autographa californica nucleopolyhedrovirus (AcMNPV) *ac83* is a baculovirus core gene and functionally unknown. In the present study, an *ac83*-knockout AcMNPV (vAc83KO) was constructed to investigate the role of *ac83* in the AcMNPV life cycle. No budded virion was produced in vAc83KO-transfected Sf9 cells, though the viral DNA replication was unaffected. Electron microscopy found that nucleocapsid assembly was aborted due to the *ac83* deletion. Domain mapping studies revealed that expression of Ac83 amino acid residues 451 to 600 partially rescued the ability of Ac83 to produce infectious budded virion. Bioassay showed that deletion of the chitin binding domain of Ac83 resulted in the failure of oral infection of AcMNPV to *Trichoplusia ni* larvae but remained infectivity by intrahemocoelic injection, implying that the domain was involved in the binding of occlusion-derived virion to the peritrophic membrane and/or other chitin-containing insect tissues. It has been demonstrated that Ac83 is the only component with chitin binding domain of the *per os* infectivity factor complex on the occlusion-derived virion envelope. Interestingly, a functional inner nuclear membrane sorting motif, which may facilitate the localization of Ac83 to the envelope of occlusion-derived virions, was identified by using immunofluorescence analysis in this study. Taken together, we demonstrated that Ac83 plays an important role in nucleocapsid assembly and establishment of oral infection.

SYMPOSIUM (Cross divisional)
Wednesday, 13:30-15:30

Trait stability and improvement

Contributed paper. Wednesday, 13:30. **114**

Trait stability and improvement of bacterial insecticides

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The bacterium, *Bacillus thuringiensis* (Bt) consists of a wide variety of subspecies, many of which are insecticidal for lepidopteran, coleopteran, or dipteran insect larvae. Subspecies such as the HD-1 isolate of *B. t.* subsp. *kurstaki* (Btk) have been used with remarkable safety for more than forty years to control lepidopteran pests in agriculture and forestry, and over the past thirty years, *B. t.* subsp. *israelensis* (Bti) has proven to be a safe and effective larvicide for control of mosquito and black fly larvae. Trait stability in commercial products is achieved by periodic mass production of potent strains, testing for efficacy, and division and storage of these to avoid losing traits through sub-culturing. Basic studies of Bt revealed that synthesis of the endotoxin crystal proteins that cause the initial toxicity,

which eventually leads to insect death, is under control of several genetic elements including strong promoters, 5' and 3' stem-loop structures, relatively short oligomers that stabilize ribosome binding of transcripts, and different "helper" proteins that assist proper folding and crystallization. By recombining these elements with one or more endotoxin proteins from Bt and *B. sphaericus* (Bs) the toxicity per unit fermentation medium in certain recombinants has been increased by as much as 10-fold. For example, driving *cry3Aa* expression with the three *cyt1A* promoters combined with the STAB sequence increases yields over wild type strains by 10-fold. Using similar constructs to produce Cyt1A, Cry11B and the Bs binary toxin yielded strains as much as 12-fold more toxic than Bti or Bs. Studies show that protein structure is an important factor in achieving high yields and high toxicity.

Contributed paper. Wednesday, 14:00. **115**

Trait Stability Among the Entomopathogenic Fungi

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Genetic trait stability is of great importance as fungal strains are developed and commercialized. In this presentation I will discuss what is known about the genetic stability of the entomopathogenic fungi, particularly the Ascomycetes.

Contributed paper. Wednesday, 14:30. **116**

Trait stability and improvement in entomopathogenic viruses: lessons learnt from baculoviruses

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Baculoviruses are the most important and the only commercially available viruses used in the biological control of insect pests. They are non-toxigen and kill susceptible host larvae within several days to weeks. Baculoviruses have been demonstrated to be environmentally friendly and safe to non-target organisms, including humans. A prerequisite for their development and registration as biocontrol agents is their genetic stability given by their double-stranded DNA genome, which is less prone to mutations than RNA viruses. A slow speed of killing and a narrow host range were sometimes considered as an economic disadvantage when compared to chemical pesticides. Hence, attempts have been made to improve the efficacy of different baculoviruses. This has been achieved in part by natural selection and by genetic engineering. In laboratory and field experiments several genetically modified baculoviruses showed some improved efficacy. However, because of neglected needs of growers, reluctant societal acceptance and lacking economic competitiveness, none of these constructs made it to the market. Recently, the emergence of field populations of codling moth resistant to *Cydia pomonella* granulovirus (CpGV) products have required to improve CpGV strains to overcome resistance. Indeed, this has been achieved by natural selection on the basis of existing biodiversity of CpGV, exemplifying the continued need of research in product development. Despite a steadily growing economic success of baculoviruses, funding in baculovirus research and scientific interest declined in recent years. However, there are important fields of research, which may facilitate the future application of baculoviruses as biocontrol agents. These include: (i) the identification, biological and molecular characterization of baculoviruses; (ii) the exploitation of the genetic diversity of baculoviruses; (iii) a better understanding of the population dynamics and virus host interactions on a population level. Research in these directions will improve the implementation of baculovirus products in integrated pest management (IPM) measures.

Contributed paper. Wednesday, 15:00. **117**

Trait stability and improvement in entomopathogenic nematodes

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Entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* can be important regulators of natural insect populations, and are used commercially as biological control agents for pest suppression. Successful biocontrol applications depend on the introduced organism having an array of beneficial traits such as virulence, host-finding, reproductive capacity etc. Thus biocontrol potential can be improved by enhancing these beneficial traits. Approaches to strain improvement include discovery, selection, hybridization, transgenics or a combination thereof. These methods have been successfully applied to a number of entomopathogenic nematode species. In addition to enhancing traits, trait stability is another factor that is critical for biocontrol success. Beneficial traits can deteriorate during repeated subculturing in laboratory or industrial settings. Deterioration of various traits has been reported in entomopathogenic nematodes. The cause of trait change was found to be genetically based (at least in part) and inbreeding depression was implicated as a significant contributing factor. Recently the creation of homozygous inbred lines was found deter the negative repercussions of trait change during serial culture. Inbred lines can be generated in the laboratory through serial self-fertilization (heterorhabditids) or sibling mating (steinernematids). Additionally, for heterorhabditids only, multiple inbred lines can be automatically generated in liquid culture because the nematodes cannot mate in the liquid media. Generation of multiple versus single inbred lines for commercial development each has advantages and disadvantages. Nonetheless, selected inbred lines with stabilized beneficial traits offer a substantial advancement in biocontrol potential for entomopathogenic nematodes.

CONTRIBUTED PAPERS
Wednesday, 13:30-15:30

Diseases of Beneficial Invertebrates 2

Contributed Papers. Wednesday, 13:15. **118**

Planning a "Needle in a Haystack" project – The search for the alternate, obligate host(s) of the zebra mussel parasite *Haplosporidium raabei*

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Who said zebra mussels were all bad? Although oysters are marine and zebra mussels are freshwater organisms, studying the latter, much-maligned, striped, invasive bivalves may well provide insight into haplosporidian diseases that have inflicted serious economic losses to the oyster industry. The life cycle of *Haplosporidium nelsoni*, as well as all other haplosporidian species, remains unknown, but there is general scientific agreement that haplosporidian species probably have complex life cycles involving one or more alternate, obligate hosts. Research efforts to prove this hypothesis and demonstrate the existence of such alternate hosts in oyster habitats, e.g., typically large estuaries and bays, have been hampered both by the expensive effort required to sample such large aquatic habitats and also the very high biodiversity, i.e., thousands of species of possible alternate hosts needing to be screened. But what if a haplosporidian disease existed in a water body that was relatively small – a water body that was easier to

sample and one with a much lower biodiversity of candidate alternate hosts to screen – possibly just hundreds, not thousands, of species? Such a rare research opportunity now exists in the Meuse River in France. *Haplosporidium raabei* was described last year from the Meuse's zebra mussel population, and a hot spot of infection has been determined to exist at a small lock on this river. Specific *in-situ* hybridization and PCR assays – molecular tools particularly valuable in detecting non-patent infections – are being developed for use in this upcoming study to define the alternate host(s) and elucidate the complete life cycle of *H. raabei*. Such a "Needle in a Haystack" project is no doubt daunting, but with success, this freshwater project could help marine scientists narrow their search for the alternate host(s) of *H. nelsoni*, as well *as* other economically important haplosporidian parasites of bivalves.

Contributed Papers. Wednesday, 13:45. **119**

Characterizing infection responses in the Pacific white shrimp, *Litopenaeus vannamei*

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The Pacific white shrimp, *Litopenaeus vannamei*, is a high production, high value species of crustacean that is subjected to semi-intensive or intensive culturing practices that facilitate disease outbreaks with significant economic consequences. To understand the infection response repertoire in this animal, we undertook a next generation sequencing effort focusing on the lymphoid organ, which we reasoned would be inherently enriched for immunity-related genes. Lymphoid organ samples were collected and RNA extracted from uninfected shrimp, and from shrimp infected with White spot syndrome virus (WSSV) or Infectious myonecrosis virus (IMNV). Libraries were generated to represent both normal-length and short RNAs by size-selection, then single-end sequenced using an Illumina Hiseq 2000. For IMNV infected and control animals, more than 110 million reads were generated, and more than 81 million reads were generated to represent WSSV infection. The repertoire of sequences reveals canonical immunity genes as described in this and other arthropods. Of particular interest is profiling the production of genes and break down products from the small RNA regulatory pathways because RNA interference is a key antiviral response. From IMNV-infected animals, microRNAs and small RNAs were identified and both were more diverse and production more abundant in uninfected vs. IMNV-infected animals. Further, characteristic siRNA pathway sequence fragments from the virus genome were evident, most of which were 22 nt in length; this allowed us to identify hot spots for virus degradation in the IMNV genome. Surprisingly, this sequence dataset also revealed 662 novel nucleotides in the IMNV genome itself.

Contributed Papers. Wednesday, 14:00. **120 STU**

Does the Common shore crab (*Carcinus maenas*) show resistance to White Spot Disease?

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The European shore crab (*Carcinus maenas*) has been shown to display a lower susceptibility to White Spot Syndrome Virus (WSSV) when compared to other European decapod species. Despite showing signs of infection with WSSV, the shore crab appeared resistant to the development of disease and was highlighted as a possible asymptomatic carrier of the virus. In this study we compared *Carcinus maenas* individuals which had been injected with WSSV and then exposed to varying temperature stress conditions. Crabs were sampled from the same geographic location so it was assumed they were from similar genetic stock. Assessment of total viral load per mg of crab tissue and histopathology from gill, connective tissues and heart suggested that individual crabs exhibited different disease development between individuals and this did not appear to be affected by temperature stress conditions. Analysis of response to WSSV exposure suggested that crabs could be divided into two groups according to differences in pathogenesis (histopathology) and relative viral replication (viral copies mg^{-1} tissue). High responders displayed elevated viral copy number mg^{-1} tissue (compared to initial dose) by the end of the study period and developed pathognomonic signs of WSSV infection within target tissues. Low responders displayed reduced viral copy number mg^{-1} tissue (compared to initial dose) by the end of the study period and did not show pathognomonic signs of WSSV infection within target tissues. The presence of both high- and low-responders in both the 'stressed' and 'non-stressed' exposure groups, and the non-significant relationship between viral copy number in individual crabs and their exposure group suggests that the response type was not dependent on the presence or absence of an external stressor but was more likely an inherent capacity within individual crabs.

Contributed Papers. Wednesday, 14:15. **121**

***Apicystis bombi*, a protozoan parasite of bumblebees, acts as an emergent infectious disease**

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The only native bumblebee, *Bombus dahlbomii*, is disappearing in the temperate forests of south Argentina and Chile. It has been hypothesized that *Apicystis bombi*, a neogregarine parasite, has recently spilled over from introduced bees and is facilitating current losses of native *B. dahlbomii*. Indeed if this parasite is introduced in novel host populations it could lead toward emergent diseases and epidemics resulting in rapid decline of *B. dahlbomii*. Until now it was impossible to molecularly characterize *A. bombi* haplotypes. Therefore it was difficult to prove that *A. bombi*, recently identified in Argentina, was introduced or already present before introduction of non-native pollinators like the European honeybee (*Apis mellifera*), and the two bumblebees *Bombus ruderatus* and *Bombus terrestris*. By use of molecular markers, the internal transcribed spacer 1 and 2, we studied within species genetic variability to identify the origin of *A. bombi* in Argentina. Only one cluster of *A. bombi* haplotypes was found in Argentina, with its most abundant haplotype identical to the most abundant one in Europe, and with a minimal structuring between Argentina and Europe (only 15.2 % of the genetic variation is explained by location). Although our data does not tell anything about the direction(s) of transmission it proves that for *A. bombi* no geographical separation is present and *A. bombi* is acting as an emergent infectious disease. Furthermore, we show a random transmission of *A. bombi* between the honeybee and the two *Bombus* species, proving this parasite lives in a multiple-host network.

Contributed Papers. Wednesday, 14:30. **122**

Dicistroviruses in bumblebees: pathology and eradication

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Israeli acute paralysis virus (IAPV), together with Acute bee paralysis virus (ABPV) and Kashmir bee virus (KBV) constitute a complex of closely related dicistroviruses. They share a similar pathology, all being lethal after injection in honeybees. In hives they are normally present in low titer, as persistent infections. But under certain environmental stressors, such as *Varroa destructor* infestation, they can rapidly re-emerge as a virulent overt infection, causing colony losses. Dicistroviruses have also been reported in non-*Apis* hymenopteran pollinators such as bumblebee species, which can become infected when placed in the neighborhood of infected honeybee hives. In this project we artificially infected *Bombus terrestris* with KBV and IAPV, both orally and through injection. Whereas high virus titers were needed, up to $1x10^7$, to establish oral infections, infection through injection was lethal at low titer. KBV infected bees placed in microcolonies combined with a short food stress period had significant lower colony startup and produced fewer offspring compared to non-infected microcolonies. Within this context we evaluated how gamma-radiation can prevent viral infections in bumblebees.

Contributed Papers. Wednesday, 14:45. **123 STU**

The interaction between IAPV and bumblebee's RNAi and JAK-STAT pathways based on qPCR analysis

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Israeli acute paralysis virus (IAPV) from the family Dicistroviridae has a widespread prevalence and is extremely virulent after artificial injection in bee hosts. RNA interference (RNAi) and JAK-STAT pathways are important defence mechanisms in invertebrates; in addition, it is known that insect hosts like *Drosophila* and mosquito recruit cross-talk between RNAi and JAK-STAT. In order to better understand the interaction of IAPV with its hosts, specifically *Bombus terrestris*, it is interesting to look at the expression changes of three core genes in the RNAi pathway (SID, Dicer-2, and Argonaute-2), the possible cross-talk gene Vago, and two genes in the JAK-STAT pathway (hopscotch and vir-1) in IAPV-infected *B. terrestris* by qPCR. Considering there are no reliable reference genes for bee-virus interaction experiments, we firstly evaluated five candidate reference genes, namely elongation factor-1 alpha (ELF1A), peptidylprolyl isomerase A (PPIA), 60s ribosomal protein (RPL23), TATA-binding protein (TBP) and polyubiquitin (UBI) in low and high IAPV-infected levels of *B. terrestris*. PPIA was indicated as an optimal reference gene in IAPV-infected *B. terrestris*. Secondly, by using PPIA as reference gene, we analyzed the six abovementioned core genes from the RNAi and JAK-STAT pathways. In essence, we detected a significant up-regulation of Dicer-2 in bumblebees with high IAPV infection level, suggesting the involvement of RNAi in the defence against the replication of IAPV in bumblebee. The expression changes of the other core genes are also discussed. In conclusion, our results lay out the initial information for the interaction between IAPV and the bumblebee's immune system.

Contributed Papers. Wednesday, 15:00. **124**

An investigation of oomycetes infecting rotifers in Brooktrout lake, NY – Lethal parasites that produce outgrowths with distinctive morphologies but of unknown function

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During examination of preserved plankton samples from Brooktrout Lake, a remote water body in the Adirondack Mountains of New York, three species of rotifers – *Keratella taurocephala*, *Polyarthra vulgaris*, and *Ploesoma truncatum* – were observed with elongate, tubular outgrowths projecting from their bodies. Light microscope examination revealed that the body cavities of these rotifers were infected with oomycete parasites, and 18S ribosomal RNA gene sequencing indicated two undescribed species were involved in these infections: one specific to *K. taurocephala* and the other specific to *P. vulgaris* and *P. truncatum*. Laboratory observations revealed that the saccate oomycete thalli within the rotifer body cavity gave rise to the outgrowths by penetrating out through the rotifer body wall within 24 h of host death. Field sampling indicated that: (1) infection with at least one of the oomycete species could be detected each year in the rotifer community; (2) infection was more likely to occur at times of high host population density and was typically accompanied by host population decline. Each of the two oomycete species had outgrowths with a distinctively different morphologies. Although aquatic rotifers are documented as hosts for a wide variety of parasites, such elongate, unbranched, rigid, tubular outgrowths as observed protruding from these Brooktrout Lake rotifers have never been previously reported. The possible adaptive value of theses outgrowths is reviewed, and we speculate that the expanded size of infected rotifer cadavers created by these outgrowths may slow their descent in the water column and deter their predation, thereby increasing the likelihood of continued propagation of oomycete infection in the lake's rotifer populations.

CONTRIBUTED PAPERS
Wednesday, 13:30-15:00

Microsporidia 1

Contributed paper. Wednesday, 13:30. **125**

A new isolate of *Nosema* sp. (Microsporidia, Nosematidae) from *Malacosoma disstria* (Lepidoptera, Lasiocampidae).

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A microsporidium closely resembling *Nosema disstriae* at the light microscopy level was isolated from forest tent caterpillar, *Malacosoma disstria* populations in southern Ontario, Canada. Mature spores were long-oval and measured 4.4 ± 0.35 (4.0-5.2) X 2.1 ± 0.14 (1.8-2.5) µm (n=40) on fresh smears. Ultrastructure of the spores was characteristic for the genus *Nosema*: 12–14 polar filament coils, posterior vacuole, and a diplokaryon. The SSU rRNA sequence identity of *Nosema* sp. MD to that of other *Nosema* species suggested *Nosema* sp. MD was most closely related to *Nosema* sp. SC (*Philosamia cynthia ricini*; 100%), *Nosema* sp. ETC-M-4-2-04 (eastern tent caterpillar; 100%) and *N. disstriae* (forest tent caterpillar; 1111/1115 (99%). In the ITS region however, *Nosema* sp. SC, and *N. disstriae* share 487/515 (95%) and 496/511 (97%) sequences identity respectively with the new isolate *Nosema* sp. MD. Intuitively, our analysis show that *Nosema* sp. MD is not identical but closely related to *N. disstriae* and both isolates infect *M. disstria*. The organization of ribosomal RNA gene sequence of *Nosema* sp. MPB show a 5'-LSU rRNA-ITS-SSU rRNA-IGS-5S-3' arrangement which is an important feature of the ''true'' *Nosema* group with type species, *Nosema bombycis*. Phylogenetic analysis based on the LSU-ITS-SSU rRNA gene sequences indicated that *Nosema* sp. MD cluster with the *N. bombycis* group and is correctly assigned as a ''true'' *Nosema* species. Ecological insights for the presence of *N. disstriae* and

Nosema sp. MD in southern Ontario and only *N. disstriae* isolated in northern Ontario *M. disstria* populations will be discussed.

Contributed paper. Wednesday, 13:45. **127**

Comparative genomics of marine microsporidia

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Microsporidia are important model cells for understanding minimilisation of the eukaryotic genome as they have the smallest number of encoded proteins of all studied eukaryotes. Across the phylum, there is an approximate tenfold variation in genome size from 2.3 Mb to 24 Mb. Recent analyses have shown that most microsporidia share a core predicted proteome with variations lying mainly in number transposon and expansion of particular gene families. This talk will compare genomes of unrelated marine microsporidia recently sequenced in the lab, and discuss which components of their genomes and proteomes vary. It will examine patterns of loss and retention of different biochemical pathways and explore patterns of expansion of particular areas of the genome, including large protein families and relate these to differences in evolutionary history, host type and lifestyle.

Contributed paper. Wednesday, 14:00. **128**

Microsporidia play the "bad guys" in a biological control program

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The hemlock woolly adelgid (HWA), *Adeges tsugae*, is an invasive hemipteran herbivore that is devastating hemlock stands in the eastern US where few natural enemies are present to suppress populations. The steady destruction of these shade tolerant evergreens is changing the structure and ecology of many eastern forests. The US Forest Service and its research partners have cooperated since the 1990s to develop a biological control program for HWA. A major focus is the evaluation and mass rearing of predatory beetles imported from China and Japan, and from the Pacific Northwest. Problems with mass rearing initiated evaluation of beetle colonies in a number of rearing facilities. Microsporidia were implicated as mortality factors in a number of colonies and in some colonies reached high prevalence levels, up to 50%, within 2-3 years. At least three species of microsporidia were recovered, two from the coccinellid *Sasajiscymnus tsugae* from Japan, and one from the derodontid *Laricobius nigrinus*, a native predator of the western North American HWA lineage. Laboratory host specificity tests of *Tubilinosema* sp. showed that three other beetle species, all putative biological control agents of HWA, were susceptible to infection, including the coccinellid *Scymnus sinuanodulus*, the native *L. nigrinus*, and *Laricobius osakensis*, native to Japan. In addition, we found microsporidian infections in *S. tsugae* and *L. nigrinus*, as well as a different microsporidian species in the native pine bark adelgid predator *Laricobius rubidus* in eastern release sites. Infection prevalence was low in release sites for two seasons of monitoring but microsporidia can seriously compromise mass rearing efforts as well as compromise the success of field-released predators.

Contributed paper. Wednesday, 14:15. **129**

Quantitative PCR-based method for detecting Nosema bombycis in silkworm egg

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Diagnosis of nosema bombycis spore in newly hatched larva by standard light microscopy procedures is used as corrective inspection for pebrine disease in silk-producing countries such as China. This method has been used for decades, however it is time-consuming, labor-intensive and insensitive. We developed a novel real-time PCR based assay that directly detect N. bombycis in silkworm eggs. Total DNA was isolated from silkworm eggs laid by infected mother moth. Extracted DNA was amplified by the LightCycler480 (Roche) PCR assay using a pair of primers to the small-subunit(SSU) rRNA gene of N. bombycis. Spore levels were estimated according to standard curve generated from serial dilutions of recombinant plasmid DNA. Through optimization of PCR reaction system, linear range of the detection method was determined, and specificity and repeatability of the method were evaluated. The results show that during the silkworm egg incubation phase, both the detection rate and spore levels are gradually increased. Thus, the bluishing stage of silkworm egg is an optimal time for pebrine inspection. The established quantitative PCR detection technology is highly sensitive, and able to detect the vegetable N. bombycis spores. The assay is readily adaptable for assessing the quality of silkworm eggs and pebrine surveillance.

Contributed paper. Wednesday, 14:30. **130**

The effects of two microsporidian pathogens on the convergent lady beetle, *Hippodamia convergens*

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Convergent lady beetles, *Hippodamia convergens* Guérin-Meneville are commercially available for biological pest control in North America. Beetles are collected from the Mountains in California where they are known to host the microsporidium *Tubulinosema hippodamiae*. The practice of using field-collected convergent lady beetles for biological pest control may result in redistribution of the pathogen when beetles are released. Microsporidia are known to infect more than one host under laboratory conditions, and although the distribution of lady beetle species often overlaps in in nature, little is known regarding the effects of more than one microsporidian pathogen on host fitness. In this study, egg cannibalism was used to examine the effects of the microsporidium *T. hippodamiae* and an undescribed microsporidium from the two-spotted lady beetle, *A. bipunctata* L. (alone and in combination) on *H. convergens* fitness (larval development and mortality and adult sex ratio).

SYMPOSIUM (Nematodes & NEMASYM)
Wednesday, 16:00-18:00

Symbiont contributions to nematode fitness

Symposium. Wednesday, 16:00. **131**

***Drosophila* transcriptional response to infection by *Heterorhabditis* nematodes and their mutualistic *Photorhabdus* bacteria**

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The *Drosophila* immune system activates a variety of immune responses against microbial infections. However, the fly immune factors and mechanisms against nematode infections are currently unknown. The nematode *Heterorhabditis bacteriophora* is an insect parasite that forms a mutualistic relationship with the gram-negative bacteria *Photorhabdus luminescens*. Following infection, *Heterorhabditis* nematodes release *Photorhabdus* bacteria that quickly multiply within the insect and produce several toxins that eventually kill the host. Although previous research has shown that the insect immune system interacts with *Photorhabdus*,

information on immune interactions with *Heterorhabditis* is lacking. We have initiated a study to identify the number and nature of *Drosophila* genes that are regulated upon infection with *Heterorhabditis* and *Photorhabdus*. We have used next generation RNA-sequencing to analyze the transcriptional profile of wild-type adult flies infected by axenic *Heterorhabditis* nematodes (worms lacking *Photorhabdus* bacteria), symbiotic *Heterorhabditis* nematodes (worms carrying *Photorhabdus* bacteria), and *Photorhabdus* alone. We have obtained around 54 million reads from the different infection types. Preliminary analysis of the data shows that *Photorhabdus* infection induces several recognition and antibacterial effector genes in *Drosophila*. Interestingly, *Heterorhabditis* infection regulates fly genes that are involved in lipid homeostasis and metabolism. Our data provide valuable information on the molecular events that take place in *Drosophila* upon infection with the two pathogens, either separately or together. Such large-scale transcriptomic analyses set the stage for future studies aimed at identifying the function of key molecules that participate in the *Drosophila* anti-nematode immune defense.

Symposium. Wednesday, 16:30. **132**

A systems biology level analysis of human host adaptation of the nematode symbiont *Photorhabdus asymbiotica.*

Jay Mulley[1], Mike Beeton[2], Nina Ockenden[2], Paul Wilkinson[3], Helge Bode[4] and Nick R. Waterfield[1]
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We have used powerful post genomic techniques to understand how the nematode symbiont *Photorhabdus asymbiotica* can infect both insects and humans. Comparative genomics, functional genomics, RNAseq digital transcriptomics, proteomics and pheno-array studies have provided detailed insights into how *P. asymbiotica* achieves this duel pathogenic state. In addition to allowing us to construct a systems level model of gene expression in different hosts, it has provided an excellent discovery platform for bioactive proteins and small drug like secondary metabolites. Such bioactive molecules include insecticides, antimicrobials and immune modulatory molecules. Studying the expression of these has allowed us to begin to unravel how this pathogen can combat the innate immune systems of both insects and people. Furthermore our network model analysis suggests that temperature adaptation and changes in metabolic activity are crucail to this host shift from insect to man.

Symposium. Wednesday, 17:00. **133**

Natural biology of antimicrobials in symbiotic *Xenorhabdus* species

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Antimicrobials involved in interspecies competition include small molecule antibiotics that are generally active against less related species and bacteriocins that are active against closely related species and strains. The symbiotic bacterium, *Xenorhabdus bovienii* (*Xb-Sj*) isolated from its entomopathogenic nematode partner, *Steinernema jollieti*, produces a R-type phage-derived bacteriocin (xenorhabdicin) encoded by the gene cluster, *xbp*1. *Xb-Sj* xenorhabdicin was shown to be highly active against a potential competitor species, *Xenorhabdus szentirmaii* (*Xsz-Sr*). *Xsz-Sr* unexpectedly produced antibiotics, but not xenorhabdicin, that were highly against *Xb-Sj*. These findings provided a novel opportunity to study the relative contributions of R-type bacteriocin and small-molecule antibiotics in reciprocal interspecies competition. To address this question we created mutant strains of *Xb-Sj* in which xenorhabdicin production was eliminated. In competition experiments in laboratory medium *Xsz-Sr* outcompeted both wild type and mutant strains of *Xb-Sj* suggesting antibiotic production determined the outcome of the competition. In co-injection competitions performed in insect hosts the wild type *Xb-Sj* strain was dominant over *Xsz-Sr*. In contrast, the mutant strains were eliminated in competition with *Xsr-Sj*. Furthermore, aposymbiotic *S. jollieti* reproduced normally in insects co-injected with the wild type *Xb-Sj* strain and *Xsz-Sr* but reproduced poorly in insects co-injected with the mutant strains. These findings indicate that *Xb-Sj* xenorhabdicin production confers a competitive advantage and enhances nematode fitness *in vivo*. To our knowledge this is the first study to characterize the role of R-type bacteriocin and small-molecule antibiotics in competition under natural biological conditions.

Symposium. Wednesday, 17:30. **134**

Carrying the Right Symbiont: How Nematode Competitive Success is Influenced by Bacterial Interactions.

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Symbionts can have dramatic effects on the fitness of their hosts. As symbionts can alter the fundamental niche of their host, they can also indirectly influence competitive interactions between host species. Symbionts may also function as mediators of interference competition, thereby more directly altering competitive interactions between host species, and potentially playing a key role in maintaining coexistence between two host species. We examined the competitive interactions between two locally sympatric species of entomopathogenic nematodes (*Steinernema* spp.). Multiple isolates of each nematode species were collected along a 240 m transect and from each their bacterial symbionts (*Xenorhabdus* spp.) were isolated and tested for their ability to interfere with each other's growth via bacteriocins (bacteriocidal toxins noted for their ability to kill closely related bacteria). Additionally we assessed each nematode isolate (with its naturally associated bacteria) for its ability to effectively colonize and quickly kill an insect host. We found that faster killing nematode isolates had a competitive advantage; however, there was no correlation between insect death rates induced by each nematode isolate and its corresponding bacterial isolate, indicating the importance of the nematode and the symbiotic context to understanding species interactions of entomopathogenic nematodes. Bacterial symbionts did favorably affect the competitive outcome of their nematode hosts when they were able to inhibit the bacteria of their competitor. Thus, the combination of nematode/bacterial traits that led to competitive success depended on which isolates were paired, suggesting that variation in competitive interactions may be important for maintaining species coexistence in this community.

CONTRIBUTED PAPERS
Wednesday, 16:00-17:45

Microbial control 2

Contributed paper. Wednesday, 16:00. **135 STU**

Temperature, dose and coverage effects on fungal biocontrol of malaria vectors

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Malaria kills more than 650,000 people each year, primarily in developing nations. Contemporary control efforts focus on suppressing mosquito vector populations with chemical insecticides applied either as indoor residual sprays or as insecticide impregnated nets. Unfortunately, insecticide resistance threatens to undermine these strategies. Fungal biopesticides, which kill mosquitoes by different mechanisms to conventional chemicals, offer a promising alternative. **Both fungal kill rate and the malarial incubation period depend strongly on temperature, but to date, no comprehensive effort has been undertaken to examine fungal virulence in mosquitoes across the thermal range relevant for malaria transmission.** To address this knowledge gap, we evaluated the virulence of *Beauveria bassiana* fungus against *Anopheles stephensi* mosquitoes at temperatures from 10-34°C. We found that, regardless of temperature, fungal exposure resulted in greater than 95% mosquito mortality prior to the end of the predicted *Plasmodium falciparum* malaria incubation period. We then utilized a theoretical model to examine the effect of varying spray

coverage levels on control. By treating nightly probability of fungal infection as a proxy for coverage, we found that, even at low doses and minimal coverage levels (10%), the fungus significantly decreased malaria transmission potential. These results suggest that fungal biopesticides may be a potent tool for malaria control in a broad spectrum of thermal environments. Furthermore, because mortality rate was sensitive to fungal dose, fungal applications could easily be adjusted to minimize selection for resistant phenotypes.

Contributed paper. Wednesday, 16:15. **136 STU**

Persistence and efficacy of *Beauveria bassiana,* against the house fly, *Musca domestica* L. on typical structural components of poultry houses

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Biopesticides comprising entomopathogenic fungi are being investigated as alternatives to chemical pesticides for control of flies in egg and poultry production facilities. One standard approach for delivering insecticides into poultry houses is as indoor residual sprays. For this type application, persistence of the spray residue is a critical factor determining long-term efficacy. Here we investigate how conidial longevity is influenced by an interplay of biotic and abiotic factors. Oil-formulated conidia of *Beauveria bassiana* were sprayed onto a range of typical structural components of poultry houses and contractor grade plastic sheeting as a potential wall-coating material, and examined for persistence and efficacy with repeated fly exposures at 1, 7, 15 and 30 days post-spray application. In the absence of flies, conidia remained viable on these surfaces for up to 3 months. Short-term exposure of flies to these treated surfaces 1 day after application resulted in 100% fly mortality within 6-10 days. However, efficacy declined rapidly following subsequent exposures, with virtually no fungal infection apparent in flies exposed on days 15 or 30. Investigating this apparent disconnect between baseline conidial viability and residual efficacy revealed that house flies both deactivate and remove fungal conidia from treated surfaces in a density dependent manner. The implications of this rapid loss of residual activity for the biopesticide technology are discussed.

Contributed paper. Wednesday, 16:30. **137**

Preventative solutions for whitefly on seasonal poinsettia cuttings

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Historically, poinsettia cuttings shipped into Ontario from offshore production facilities have carried very low levels of immature Bemisia (eggs and nymphs). These have been successfully controlled by preventative releases of parasitoids (*Encarsia formosa*, *Eretmocerus mundus*). In 2012, though, cuttings arrived into Ontario carrying large numbers of Bemisia eggs and nymphs. Although parasitoid releases proceeded as normal, they failed to regulate whitefly populations and multiple pesticide treatments were required. However, endemic whitefly resistance (owing to heavy pesticide pressures in offshore production facilities) means that pesticides registered in Canada frequently have reduced efficacy. To ensure greater sustainability in poinsettia production, new methods of control are required that can be applied to cuttings to prevent pest populations developing beyond the 'capacity' of the parasitoids used, and to ensure that effective biological control systems can be maintained through the crop production cycle. Several biopesticide treatments, applied to infested cuttings by dipping immediately prior to sticking, have been tested to assess their relative effectiveness against whitefly, ensure compatibility with parasitoids, and that they are not phytotoxic. The project has allowed effective treatments to be identified that can be readily implemented a commercial scale.

Contributed paper. Wednesday, 16:45. **138**

Commercial formulation of *Metarhizium anisopliae*-based biopesticide (Campaign®) reduces damage by *Maruca vitrata* on cowpea and increases grain yield

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The Legume pod borer, *Maruca vitrata,* is a devastating insect pest of cowpea causing yield losses of 20-80% in many sub-Saharan African countries. Current control measure relies heavily on the use of synthetic pesticides. In an effort to develop an environmentally acceptable alternative control strategy, 20 isolates of *Metarhizium anisopliae* and *Beauveria bassiana* were screened against first-instar larva. Two *M. anisopliae* isolates (ICIPE 18 and ICIPE 69) were identified as the most effective in terms of overall mortality and speed of kill. Evaluation of their performance in different liquid media for mass production revealed that ICIPE 69 produced significantly higher concentration of propagules in Jenkin-Prior (2.6±0.4 x 10^8 propagules ml^{-1}) and APU1 (2.4±0.7 x 10^8 propagules ml^{-1}) media compared with ICIPE 18 (Jenkin-Prior: 1.5±0.2 x 10^6 propagules ml^{-1}; APU1: 5.2±1.7 x 10^7 propagules ml^{-1}). On the basis of these results, commercial formulation of *M. anisopliae* ICIPE 69 marketed as Campaign® in Africa was field-tested for the management of *M. vitrata* on cowpea in Coastal region, Kenya. Campaign® was applied at the rate of 200 ml ha^{-1} (~ 1 x 10^{13} conidia ha^{-1}) and compared with Neem (Nimbecidine) and Karate (Lambda-cyhalothrin). Compared with the control, the biopesticide, neem, and insecticide treatments significantly reduced plant damage by *M. vitrata.* Cowpea grain yield was 1071.2 kg ha^{-1}, 664.4 kg ha^{-1}, 1578.2 kg ha^{-1} and 323.8 kg ha^{-1} in the biopesticide, neem, Karate, and control treatments, respectively; thus, demonstrating the potential role of the biopesticide in Maruca IPM in Africa.

Contributed paper. Wednesday, 17:00. **139 STU**

Transcriptomic Analysis of Tripartite Interactions of *Metarhizium*, *Plasmodium*, and *Anopheles gambiae*

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Metarhizium is a pathogen of the human malaria mosquito *Anopheles gambiae*. We conducted a transcriptomic analysis of *A. gambiae* infected with *Plasmodium* and/or *Metarhizium* to identify differences and overlaps in the gene expression of mosquitoes when confronted with these eukaryote pathogens and to determine which components of the mosquito immune response are enhanced or suppressed by co-infection with Plasmodium and *Metarhizium*. Four populations of mosquitoes were used to maximize the comparative capacity of the study: *Plasmodium+/Metarhizium+*, *Plasmodium+/Metarhizium-*, *Plasmodium-/Metarhizium+*, and *Plasmodium-/Metarhizium-*. RNA was extracted from the decapitated mosquitoes from each population and was sequenced using Illumina RNA-seq with paired end 100 bp reads. The results will provide a comprehensive understanding of the tripartite interactions between *Metarhizium*, *Plasmodium* and *A. gambiae* that will provide context for analyzing the transgenic strains we have developed.

Contributed paper. Wednesday, 17:15. **140**

Field trials using the entomopathogenic fungus *Beauveria bassiana* for the control of UK stored products pests

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The UK grain industry yearly applies many tonnes of pesticide to UK grain stores in an attempt to control stored products pests. EU legislation is restricting the number of chemical pesticides available for use and alternative methods of control are required. Through two previous projects an isolate of *Beauveria bassiana*, obtained from *Sitophilus* sp. in the UK, was shown to give good control in the laboratory and artificial grain store arenas. Susceptible pests included *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus* and *Sitophilus granarius*. A present phase is intended to lead to registration of a product for European markets. In 2012 the first full scale efficacy trials were conducted, in small silos on a farm. These trials resulted in greater than 90 % kill of the saw toothed grain beetle, *O. surinamensis* and *C. ferrugineus*. This year further trials will be conducted.

Contributed paper. Wednesday, 17:30. **141 STU**

Field performance of *Brevibacillus laterosporus* and a commercially available *Beauveria* product against brassica pests

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Two New Zealand (NZ) strains of *Brevibacillus laterosporus (Bl)*, a spore-forming bacterium which produces crystalline inclusions, were isolated from brassica seeds. Bioassays for activity against *Plutella xylostella*, the diamondback moth (DBM), revealed 100% mortality for second instar DBM larvae within 36 hours. Similar high mortality against white butterfly larvae, *Pieris rapae*, was achieved within 5 days. Bioassays did not reveal any susceptibility of bees to *Bl*. The field performance of two NZ strains of *Bl* was tested in five preliminary field trials with brassicas. The *Bl* treatments were applied unformulated and compared to two standards, DiPel^{DF}, a commercial product of *Bacillus thuringiensis* subsp. *kurstaki*, and a formulated product (Beaublast™) of *Beauveria bassiana* containing metabolites and spores with insecticidal activity towards caterpillar and aphid species. All bio-control products significantly reduced brassica leaf damage from insect attack when compared with the untreated control, with Beaublast providing the most consistent results. The NZ strains of *Bl* and Beaublast show significant promise as biopesticides for brassica pests and could be included into sustainable integrated pest control programmes.

CONTRIBUTED PAPERS
Wednesday, 16:00-17:30 Haselton

Viruses 3

Contributed paper. Wednesday, 16:00. **142**

Characterization of a Colombian granulovirus isolated from *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae

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The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) is an important pest of maize crop in Colombia, causing around 60% of yield losses. Biological control of this pest has included the use of its nucleopolyhedrovirus SfMNPV, which has shown high potential as biopesticide. Granulovirus of *S. frugiperda* (SfGV) has been poor studied even the fact that this kind of virus is able to enhance the infectivity and virulence of NPVs. In this sense, a Colombian SfGV (VG008) was morphologically, biologically and molecularly characterized in comparison with a reference isolate from Brazil (SfGVBr). Restriction endonuclease analysis (REN) using five enzymes showed different patterns among isolates and genomes sizes were estimated at 135.1 Kb for VG008 and 132.6 Kb for SfGVBr. SfGV granules of both isolates were ovoidal shaped with an approximate size of 409~460 nm x 150~171 nm, and contained one single virion. The median lethal concentrations (LC_{50}) were of $4.5x10^5$ granules/mL for VG008 and $1.6x10^5$ granules/mL for SfGVBr. The mean time to death (MTD) values were estimated for virus concentrations that resulted in ~90% larval mortality and VG008 and SfGVBr presented similar MTD values with 29 and 33 days, respectively. Regarding ultraviolet B radiation sensitivity after 2 hours of direct irradiation, both SfGV isolates reduced the insecticidal activity, with an inactivation of 94% for VG008 and 96% for SfGVBr. Considering that GVs may enhance the NPVs activity in a coinfection process, characterized granulovirus VG008 will be used for improving a Colombian NPV biopesticide by using coinfection strategy in order to efficiently control that pest.

Contributed paper. Wednesday, 16:15. **143**

***Mamestra configurata* nucleopolyhedrovirus-B: Characterization of geographic isolates at the genome sequence level.**

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Bertha armyworm (BAW) on the Canadian prairies is a significant economic pest of canola and during peak outbreaks larval populations become heavily infected by baculoviruses. We previously sequenced the complete genomes of three *Mamestra configurata* nucleopolyhedrovirus (MacoNPV) geographic isolates and found that they constitute two different virus species, MacoNPV-A and –B. The two MacoNPV-A isolates 90/2 and 90/4 had a high degree of identity but also had several gene deletions/insertions and were significantly different with respect to virulence. Despite a large collection of MacoNPV isolates from bertha armyworm (BAW) populations over a wide geographic area of western Canada and from several temporal outbreak cycles of this noctuid pest, to date only one isolate of MacoNPV-B had been characterized. A multiplex-PCR assay was designed to distinguish MacoNPV-A and -B and used to screen a collection of single-cadaver NPV isolates from the most recent BAW outbreak (2012) in western Canada. More than 10 additional MacoNPV-B single-cadaver isolates were identified from one region and several of these have been subjected to 454 pyrosequencing and contig assembly. The genetic relatedness of these isolates to the original MacoNPV-B (96/2) and MbMNPV isolates from Asia was characterized. The major differences identified were the complement of bro genes and the region between hr1 and bro-a, which was shown to vary substantially. The infectivity and virulence of these isolates were also compared by dose-response bioassays in BAW larvae to determine possible phenotypic differences.

Contributed paper. Wednesday, 16:30. **145**

Detection of native baculovirus *Sf*MNPV in Sinaloa, and their virulence on fall armyworm.

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During 2010-2011 was conducted an intensive search of larvae of corn fall armyworm *Spodoptera frugiperda* (J. E. Smith) in a cultivate maize area in Guasave Sinaloa, México. The larvae with signs of virus infection were analyzed using PCR, with specific primers to *Sf*MNPV. The detection was positive to baculovirus (575 pb), so a sample was sent to sequencing. Were infected with the native virus the 0.75% of larvae to field level; from these a inoculum was prepare to evaluate the virulence of native *Sf*MNPV. Bioassay was realized against three instar larvae of *S. frugiperda*; to the 84hr began the infection process, with mortality of LD_{50}/mL $10^{4.59}$ (cv 0.09) and LT_{50} 111.56 (cv 0.46 h). After 132 hr all insects dead, showed typical virus infection signs. These results indicate the presence of baculovirus *Sf*MNPV in the area, and suggest possibilities for use it as a biological control agent against fall armyworm in Sinaloa.

Contributed paper. Wednesday, 16:45. **146**

Stability and life history parameters of a nucleopolyhedrovirus-resistant strain of the smaller tea tortrix, *Adoxophyes honmai*

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A strain of the tea pest *Adoxophyes honmai* (Tortricidae, Lepidoptera) that is resistant to a nucleopolyhedrovirus (NPV) was selected by subjecting a field-collected *A. honmai* population to a 70% lethal concentration (LC_{70}) of *A. honmai* NPV (AdhoNPV) in the laboratory. The selection was carried out every generation for 16 years, and after 155 generations the AdhoNPV-selected strain (resistant strain; R-strain) showed over 10,000-fold higher resistance to AdhoNPV than the nonselected strain (susceptible strain; S-strain). The inheritance of resistance by *A. honmai* against AdhoNPV is not sex-linked and may be due to a polygenic trait. The mechanism of resistance is still unknown, but viral infection was blocked both in the midgut and also in other tissues in the hemocoel. We examined the stability of resistance by removing the R-strain selection at generation 149. LC_{50} values of the strain in which selection had been stopped (RN-strain) showed no significant difference from that of the R-strain over 4 generations (from generation 152 to 155). Thus, the resistance trait was stable even after the selection was stopped. Three life history parameters for the RN-strain and S-strain were examined. Egg hatchability was not significantly different; larval period of the RN-strain was shorter than the S-strain; and pupal weight was greater in the RN-strain than in the S-strain. Therefore, no fitness cost was evident in the RN-strain. In *A. honmai*, resistance to AdhoNPV and these life history parameters may not require a trade-off, and the resistance trait may be maintained in the field.

Contributed paper. Wednesday, 17:00. **147**

Protein–protein interactions and identification of the A-spike in *Dendrolimus punctatus* cypovirus

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The atomic structures of the cypovirus (CPV) virions have been elucidated by the cryoelectron microscopy with the description of three capsid proteins. However, the RNA segment encoding the A-spike protein has not so far been identified. In order to determine which segment encodes the A-spike protein, and understand the functions of the viral structural proteins in detail, yeast two-hybrid system was employed to study interactions among the encoded proteins of 10 segments of *Dendrolimus punctatus* CPV (DpCPV). Nineteen pairs of the interactions were detected in one direction, and four pairs of interactions were identified in both directions, whilst four proteins were found to self-associate. The polyhedrin interacted with VP1, RdRp, VP2, VP4, VP5, NSP1, NSP2 and with itself. Six pairs of interactions have been identified among the three capsid proteins and VP4, five of them were detected in one direction: VP1 (capsid shell protein) and VP3 (turret protein), VP1 and VP4, VP1 and VP5 (clamp protein), VP3 and VP4, VP3 and VP5, and one of them was detected in both directions: VP4 and VP5. The interaction between VP3 and VP4 was subsequently confirmed by far-western blots, and the interaction between VP4 and polyhedrin was confirmed by His pull-down assay. Since VP4 interacts with methyltransferase domain of turret protein (VP3) and polyhedrin, it can be presumed that VP4 is the A-spike protein of CPV.

Contributed paper. Wednesday, 17:15. **148**

Molecular characterization of a novel Cypovirus isolated from *Dendrolimus punctatus*

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A cypovirus (CPV), with novel electrophoresis pattern and unique conserved terminal sequences of genomic dsRNA segments, was isolated from *Dendrolimus punctatus* using *in vivo* cloning technique. The occlusion bodies and virions of this novel *D. punctatus* CPV (DpCPV) displayed the typical polyhedral shape and icosohedral shape of CPVs under electron microscopy. Its genome contained 16 dsRNA segments with a size range of 783 to 4051 bp, and the electrophoretic migration pattern differed from the 20 CPV types reported to date. By using full-length amplification of cDNAs (FLAC) technique, the 16 dsRNA genomic segments were cloned and sequenced. Each segment encoded one ORF and possessed conserved terminal sequences of ACUUUU and UAGAGC at the 5' and 3' ends, respectively, except that the segment 15 and 16 had a terminal sequence of CCAGC at the 3' end. These conserved terminal sequences were not consistent with any of the known CPV types, thus we tentatively named it as DpCPV-21. A phylogenetic tree based on amino acid sequence of polyhedrin indicated that DpCPV-21 was most closely related to *Antheraea mylitta* CPV (AmCPV), *Antheraea assamensis* CPV (AaCPV) and *Antheraea proylei* CPV (ApCPV). Sequence homology comparisons displayed that 10 segments of DpCPV-21 were homologous to the existing CPVs, whereas no significant similarity was found for the proteins encoded by the other segments. Usually there were not more than 12 segments in the CPV genomes, and we propose that DpCPV-21 acquired heterogenous segments from its surroundings in during its evolution.

CONTRIBUTED PAPERS
Wednesday, 16:00-17:45

Bacteria 3

Contributed paper. Wednesday, 16:00. **149**

Toxin production by *Brevibacillus laterosporus*, a potential biocontrol agent of diamondback moth and other insects

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Two New Zealand (NZ) strains of *Brevibacillus laterosporus*, a spore-forming bacterium, were isolated from brassica seeds and a third strain was isolated from potatoes. All three NZ strains form crystalline inclusions. Initial bioassays revealed high insecticidal activity towards insects of several orders, particularly Lepidoptera and Diptera. Separation of crystals from spores of one of the NZ strains by gradient centrifugation showed that toxicity was strongest in the crystals. SDS-PAGE of the proteins demonstrated that the crystals are composed of proteins with sizes of approximately 200 kDa, 150 kDa and 66 kDa. Genome sequencing of the three strains identified a number of putative toxin encoding genes, including novel *cry* and *vip*-like toxins. Although these genes show some degree of homology to the genes of *Bacillus thuringiensis*, they are unique and could represent new groups within known invertebrate toxin gene families. *Cry*-like genes were individually cloned into *Escherichia coli* and will be expressed and tested in bioassays toward the diamondback moth larvae and

mosquitoes to confirm toxicity. The NZ strains of *B. laterosporus* could be used as new biocontrol agents and included into sustainable integrated pest management programmes.

Contributed paper. Wednesday, 16:15. **150**

Interactions between mosquitocidal Cry4Aa and the brush border membrane proteins of *Culex pipiens* larvae.

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Cry4Aa is a mosquitocidal protein produced by *Bacillus thuringiensis* subsp. *israelensis* that exhibits specific toxicity to *Anopheles*, *Aedes*, and *Culex* mosquito larvae. The three-dimensional structure of Cry4Aa has been determined. However, the functional structures of Cry4Aa related to its toxicity are still unidentified. In this study, the polypeptides derived from domains II and III of Cry4Aa were expressed as protein inclusions using the expression vector, p4AaCter. The polypeptides were purified using Ni-charged affinity column. Interactions between these polypeptides and the brush border membrane (BBM) proteins prepared from *C. pipiens* larvae were analyzed using quartz crystal microbalance (QCM) device. The polypeptides containing domain II β1-α8, β2-3 and domain III showed relatively high affinities to the BBM proteins and their KD were estimated as 59, 54, and 63 nM, respectively. This suggested that multiple sub-sites of Cry4Aa may work cooperatively for receptor binding. Bioassay against *C. pipiens* larvae was done using the Cry4Aa pretreated with various monosaccharides. Interestingly, enhancement of the Cry4Aa toxicity was observed by GalNAc pretreatment. On the other hand, the pretreatment with fucose inhibited the toxicity of Cry4Aa. This suggested that the sugar-side-chain derived from BBM proteins was involved in the toxicity of Cry4Aa against *C. pipiens* larvae. Thus, insecticidal mechanism of Cry4Aa may be unique and quite different from that of well-characterized other Cry toxins.

Contributed paper. Wednesday, 16:30. **151**

Evidence for lateral transfer of cereulide gene cluster by identification of a composite transposon in emetic *B. weihenstephanensis*

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Bacillus cereus has been associated with two distinct clinical types of food toxi-infection: diarrhea and emesis. Whereas different heat-labile enterotoxins have been suggested to contribute to the diarrheal symptoms, cereulide, a heat-stable toxin has now been confirmed to provoke emesis. In rare cases however, this toxin can also lead to human death. Cereulide is a cyclic dodecadepsipeptide ionophore, produced via non-ribosomal peptide synthesis (NRPS). Early studies had shown that the genetic determinants of cereulide (a 24-kb gene cluster of *cesHPTABCD*) are located on a 270-kb plasmid related to the *Bacillus anthracis* virulence plasmid pXO1, and the *B. cereus* cereulide-producing strains formed a homogeneous group. However, a recent study identified a distinct cereulide-producing group identified as psychrotolerant *B. weihenstephanensis*. Moreover, the location of the cereulide genetic determinants was shown to vary, strongly suggesting genomic mobility of the NRPS cluster. In this study, the conjugation experiments were performed to survey the potential horizontal transfer capability of the cereulide-producing plasmids from different emetic *B. cereus* group isolates, which indicated that these plasmids are not self-transmissable or mobilizable. However, the cereulide-producing *B. cereus* group strain can be the potential host for conjugative plasmid pXO16 from *B. thuringiensis*, and the resulting transconjugant has the capability for the retrotransfer of pXO16 to other recipients. Moreover, the interior and adjacent DNA sequence of the *ces* gene cluster from eight cereulide-producing strains, representing different types, were sequenced and analyzed. Sequence variation depending on different cereulide-producing group was noticed. The most striking observation was the identification of two copies of insertion elements (named *IS*ces) with a perfect 16 bp inverted repeat (IR) flanking the up- and down- stream of the *ces* gene cluster of two psychrotolerant *B. weihenstephanensis* strains, indicating the transposition origin. An IS*ces*-based composite transposon pTnKm was created to carry two copies of IS*ces* element in inward and opposite orientations flanking the Km^R marker. Transposability of pTnKm was confirmed by transposition assays in mating-out system. It was demonstrated that pTnKm can transpose efficiently in random manner in plasmid R388 (a conjugal plasmid free of transposon) and chromosome of *Escherichia coli* by Southern blot and by sequence analysis of the TnKm insertion sites. The study has explored the probably lateral genomic transfer mode of cereulide genetic determinants and provided a greater understanding of the virulence transmission of *B. cereus* group.

Contributed paper. Wednesday, 16:45. **152**

Treat worm infections with crystal protein expressing in probiotic like bacteria

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Soil-transmitted helminths (namely hookworms, whipworms, and *Ascaris* large roundworms) are intestinal nematodes, which cause diseases of poverty that infect upwards of two billion people worldwide. These parasites are a major threat to health and development of hundreds of millions of children and pregnant women. Enormous hurdles must be overcome in order to develop and deliver urgently needed new therapies (anthelmintics) to replace old ones that perform sub-optimally and are losing efficacy. Any new therapy must be extremely cheap, be able to be produced in tremendous quantities to treat hundreds of millions of people, have a stable shelf life, and be capable of storage and delivery under adverse environmental conditions. Our research has uncovered a radical and unique new approach that solves each of these challenges: expression of vertebrate-safe, anthelmintic (anti-nematode) proteins in "probiotic-like" food-grade bacteria. Such bacteria can be produced cheaply, in great quantity, stored stably, and delivered under adverse conditions. Here we will discuss our work to develop such engineered bacterial therapy using the anthelmintic crystal protein Cry5B normally made by *Bacillus thuringiensis* (Bt). We will present data on how Cry5B can be expressed in a non-Bt bacterium related to food-grade bacteria, and the strong efficacy of such a bacterium in clearing hookworm infections in rodents. We will also update progress on engineering several food-grade bacteria to express Cry5B as a critical step towards implementation of this novel anthelmintic approach.

Contributed paper. Wednesday, 17:00. **153**

Strategies to address corn rootworm control challenges

William Moar, Matthew Carroll, Graham Head, Tom Clark, and Gerrit Segers
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Corn Rootworm (CRW) *Bt* technologies containing Cry3Bb1 have been in the market for 10 years and have provided significant value to growers. However, increased damage from CRW in some fields has been observed in the last few years. Beginning in 2012, Best Management Practices (BMP's) were implemented to reduce performance inquiries in future years, as demonstrated by 2012 field results. This presentation will discuss the current status of CRW performance by Cry3Bb1 technologies in the US, and how the use of BMP's can extend the durability of current and future

Cry3Bb1 technologies, including pyramiding Cry3Bb1 with Cry34/35 and dsRNA.

Contributed paper. Wednesday, 17:15. **154**

Characterization of midgut Cadherin in Bt Cry3A-susceptible and resistant populations of the beetle *Chrysomela tremulae*

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Field screening has previously allowed to detect poplar leaf beetles highly resistant to *Bacillus thuringiensis* Cry3Aa toxin. An isofemale line of *Chrysomela tremulae* (Ct) was selected on Cry3Aa-expressing poplars. Resistance to Cry3Aa was almost completely recessive and associated with a single autosomal locus. One family of putative receptor of Cry3Aa, i.e. aminopeptidases N, is apparently not affected in resistant insects : none of the 3 identified members of this family of proteases is differentially transcribed or display any amino-acid change when compared to the susceptible sequences. Pyrosequencing of *C. tremulae* larval midgut resulted in six cDNA contigs homologous to insect cadherins. These sequences were our starting point for cloning the full sequence of midgut epithelial cadherin in *C. tremulae*. Sequence analysis indicate that Ct cadherin is 1705 amino acid-long and organized in 10 predicted repeats. As expected, this protein is very similar to other coleopteran cadherins reported so far: it shares 98% similarity with *Diabrotica vergifera*'s one (another *Chrysomela*), 97% with *Tenebrio molitor* and 94% with *Tribolium castaneum* cadherins. Interestingly it also shares high similarity with several *Lepidoptera* (90-94%) and *Diptera* (90% with *Drosophila*) cadherins. Ct Cadherin is mainly expressed in the midgut of L1 larvae and adults. No qualitative or quantitative difference was detected in the cadherin transcripts of Cry3A-resistant beetles when compared to susceptible ones. However, several non silent mutations were found in the primary sequence of the resistant allele which might be related to an alteration of the binding capacity of the toxin to its target receptor.

Contributed paper. Wednesday, 17:30. **155 STU**

Inheritance of Cry1F resistance, cross-resistance and frequency of resistant alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

[1]Ana Maria Velez, [1]Terence A. Spencer, [2]Analiza P. Alves, [3]Dan Moellenbeck, [4]Haridas Chirakkal and [1]Blair D. Siegfried

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Transgenic maize, *Zea maize* L., expressing the Cry1F protein from *Bacillus thuringiensis* (*Bt*) has been registered for *Spodoptera frugiperda* (J. E. Smith) control since 2003. Unexpected damage to Cry1F maize was reported in 2006 in Puerto Rico and Cry1F resistance in *S. frugiperda* was documented. Cry1F resistance in *S. frugiperda* represents the first instance of field failure associated with insect resistance to a *Bt* crop leading to withdrawal from the marketplace. The inheritance of Cry1F resistance was characterized in a *S. frugiperda* resistant strain originating from Puerto Rico which displayed >387-fold resistance to purified Cry1F. Inheritance experiments indicated that resistance is recessive, autosomal and conferred by a single locus. In addition, cross-resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ba, Cry2Aa and Vip3Aa was assessed in the Cry1F resistant strain. There was no significant cross-resistance to Cry1Aa, Cry1Ba and Cry2Aa, although only limited effects were observed in the susceptible strain. Vip3Aa was highly effective against susceptible and resistant insects indicating no cross-resistance with Cry1F. In contrast, significant cross-resistance (< 20-fold) was observed for both Cry1Ab and Cry1Ac. Because resistance was recessive and conferred by a single locus, an F_1 screen was used to measure the frequency of Cry1F resistant alleles from populations of Florida and Texas in 2010 and 2011. A frequency of 0.13 was found in Florida, while Texas populations had a resistant allele frequency of 0.015. Results indicate that resistance alleles exist among continental United States populations and are persistent in resistant populations (e.g. Puerto Rico).

WORKSHOP
Wednesday, 21:00-21:30

VIRUS DIVISION

Workshop. Wednesday, 21:00. **156**

Deep sequencing technology for arthropod virus discovery

Sijun Liu[1], Diveena Vijayendran[1], Bryony C Bonning[1]

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Arthropods are commonly infected with multiple viruses including sublethal, asymptomatic, and latent infections. However, conventional methods for virus isolation typically lack the sensitivity required for detection of viruses that are present in low abundance and traditional approaches have limitations for acquiring full length viral sequences. Next Generation sequencing (NGS) technologies have revolutionized virus discovery, and the study of virus prevalence. In this workshop, we will use identification of viral sequences from soybean aphid (*Aphis glycines*) and penaeid shrimp (*Litopenaeus setiferus*) to compare advantages and disadvantages of various NGS sequencing methods for virus discovery, specifically sequencing of small RNA, RNA-seq and viral RNA isolated from crudely extracted virion samples. We will also introduce bioinformatics methods used for analysis of transcriptome and small RNA data generated by high throughput sequencing technology for *de novo* assembly of viral sequences and identification of viral sequences from BLAST data. In addition, methods for generation of full-length or near full-length viral genome sequences will be discussed.

THURSDAY - 15 August

SYMPOSIUM (Bacteria)
Thursday, 08:00-10:00

Reflections on Bt mode of action

Symposium. Thursday, 08:00. **157**

Post-binding events in the mechanism of action of Bt toxins and parallels with mammalian pore-forming toxins

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Bacillus thuringiensis crystal proteins are pore-forming proteins that attack invertebrate cells. Some recent discussions have raised the issue as to whether there are mechanisms other than pores for how these proteins work. Here, we will review research on how crystal proteins attack cells. In particular, we will discuss many cellular events that are known to be triggered by crystal proteins post-binding, including signal transduction pathways, pore defense pathways, and cellular trafficking pathways. We will discuss the relevance of these cellular events towards functional cellular responses to crystal proteins. We will discuss parallels between these cellular events and effects of (non-crystal protein) pore-forming proteins on mammalian cells. Our data indicate that many of the effects of crystal proteins can be attributed to the myriad of cellular responses that occur when cells in general are subjected to pores at their membranes.

Symposium. Thursday, 08:30. **158**

Role and mechanism of pore formation by *Bacillus thuringiensis* insecticidal crystal toxins

Vincent Vachon, Raynald Laprade and Jean-Louis Schwartz
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The ability of the insecticidal toxins produced by *Bacillus thuringiensis* to form pores in the plasma membrane of midgut epithelial cells of susceptible insects, following their interaction with specific membrane proteins, has long been established and the properties of the pores have been characterized extensively. More recently, several membrane proteins have been identified as putative toxin receptors and considerable effort has been devoted to the understanding of their involvement in toxicity. A rather elaborate model has thus been put forward in which the toxin is hypothesized to interact sequentially with at least two of these receptors before inserting into the membrane. On the other hand, the importance of pore formation has been questioned and a model was proposed in which the toxins exert their effects by activating a magnesium-dependent intracellular signalling pathway. This presentation will focus on a discussion of the experimental evidence on which these current models are based as well as on the role and consequences of pore formation in the mechanism of intoxication.

Symposium. Thursday, 09:00. **159**

Learning the ABCs of Bt

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Insecticidal crystal toxins from the bacterium *Bacillus thuringiensis* (Bt) kill insects via a complex mode of action resulting in the creation of cytolytic pores in the membrane of midgut epithelial cells. Recent genetic studies in four species of Lepidoptera have found mutations in an ABC transporter in strains that have evolved resistance to Cry1A toxins. Functional studies using germline transformation in *Bombyx mori* and heterologous expression confirm the essential role of ABC proteins in Cry1A toxin mode of action. It is proposed that ABC proteins function in the insertion of toxin monomers or the toxin oligomeric pre-pore structure into the midgut epithelial membrane, a crucial step for which the mechanism has not been known in detail. Properties of ABC transporters suggest strategies to increase efficacy of Bt toxins and to delay the evolution of Bt toxin resistance in target insect pests.

Symposium. Thursday, 09:30. **160**

Structure/function studies reveal the evolution of pore-forming toxins in bacteria and mammals

Rodney Tweten
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The cholesterol-dependent cytolysins (CDCs) are pore-forming toxins that are present in a wide variety of Gram-positive pathogens and a few Gram-negative soil organisms. The CDCs contribute to the pathogenic mechanisms of many of the Gram-positive pathogens in human and animal diseases, but in many cases may also contribute to bacterial defense against predation by lower eukaryotic organisms. Structure and function studies on the CDCs have shown that they undergo a complex choreography of structural changes and interactions, which are initiated by the interaction of the CDCs with their receptors and lead to the formation of an extraordinarily large pore complex in the membrane of cholesterol-rich eukaryotic cells. More recently, the crystal structures of several membrane attack complex/perforin (MACPF) proteins have shown that a protein fold of the CDCs, which is responsible for the formation of the CDC β-barrel pore, is also present in the MACPF protein family, suggesting that these two families of toxins are ancient ancestors. The MACPF proteins are widespread in eukaryotes, and to a lesser extent in prokaryotes. Recent studies suggest that the MACPF proteins also form a β-barrel pore, but important differences appear to exist in the assembly pathway of the MACPF pore complex. The assembly pathway for the CDCs will be discussed and how these studies have led to insights into the mechanism of pore formation by the MACPF protein family.

CONTRIBUTED PAPERS
Thursday 08:00-10:00

Viruses 4

Contributed papers. Thursday, 08:00. **161**

Accumulation kinetics of eight-thymidine mononucleotide repeats of *Anticarsia gemmatalis* multiple nucleopolyhedrovirus *fp25k* during serial passage in Tn5 cells

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Serial passage of baculoviruses in insect cells often leads to accumulation of few-polyhedra (FP) mutants caused by *fp25k* mutations with the conversion of seven-adenosine mononucleotide repeats (A7 MNR) to A8. Here, we report that *Anticarsia gemmatalis* multiple nucleopolyhedrovirus (AgMNPV) *fp25k* has an A7 and a T7 MNR. During AgMNPV serial passage in Tn5 cells, the T7 MNR was mutated to T8 but not the A7 MNR, as detected by PCR sequencing of the amplified *fp25k* locus. Conversion of the T7 MNR to T8 was found as early as passage one and accumulated steadily, which correlated with the development of FP in Tn5 cells. A search of MNR sequences within various baculovirus *fp25k* revealed that the majority of baculoviruses with MNRs have A7 and that AgMNPV is the only one that has a T7 MNR in the *fp25k* gene. To understand why the T7 MNR mutated but not the A7 of AgMNPV *fp25k*, free energy of the hairpin structures of baculovirus *fp25k* MNR regions was compared. We found that free energy of the T7 MNR region is similar to the A7 MNRs of *fp25k* that have been reported to mutate to A8. Southern blot of DNA from Tn5 cells infected with AgMNPV with a radiolabeled AgMNPV probe showed streaks of smeared DNA significantly larger than the AgMNPV genome. Collectively, these data suggest that the AgMNPV *fp25k* T7 MNR mutates at a high frequency and the Tn5 cellular DNA polymerase might be involved in the rolling circle replication of the AgMNPV genome.

Contributed papers. Thursday, 08:15. **162 STU**

First functional annotation of a polydnavirus: the genome of CcBV explored.

Germain Chevignon[1]; Sébastien Cambier[1]; Julien Thézé[1]; Sébastien Moreau[1]; Elisabeth Huguet[1]; Jean-Michel Drezen[1].
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Cotesia congregata develops as a gregarious endoparasitoid into larvae of the tobacco hornworm *Manduca sexta*. The parasitoid wasp has evolved virulence strategies using an obligatory viral symbiont from the Polydnavirus (PDV) family named *Cotesia congregata bracovirus* (CcBV). CcBV particles are produced by specialized cells of the wasp ovaries and are injected along with the eggs into the host body. The PDV genome exists as two distinct forms: a linear symbiotic form and an encapsulated circular pathogenic form. The integrated genome is made up of 35 segments and encodes 222 predicted genes distributed in 34 multigenic families. In the wasp, viral gene expression mainly concerns genes involved in viral particle production. In the caterpillar host, the expression of only a few selected candidate virulence genes had been studied, and so far we lacked a global vision of viral gene expression. Here we performed a large-scale

transcriptomic analysis by *454* sequencing of two distinct immune tissues (fat body and hemocytes) of the host *M. sexta* parasitized by *C. congregata*. Following this analysis, we were able to identify 77 CcBV genes expressed 24hrs after parasitism. This analysis allows us for the first time to have a snapshot of global viral gene expression during parasitism at one given time and in two tissues. In particular, we could show differential gene expression among genes belonging to different families or different segments. This type of analysis will help us to highlight viral virulence genes that play an essential role in the host-parasitoid interaction.

Contributed papers. Thursday, 08:30. **163 STU**

***Spodoptera exigua* MNPV ORF28 blocks viral replication in cell culture.**

Amaya Serrano[1,2], Sarah Nadif[2], Monique van Oers[2], Primitivo Caballero[1], Just M. Vlak[2], and Gorben Pijlman[2]
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The US1 isolate of the *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) is composed of several genotypic variants, most of which carry deletions in the ORF15-41 region. SeUS1 viral DNA was used for the construction of bacmids by direct cloning in *E. coli*. Bacmids containing the complete SeMNPV genome (SeBac10) and a genotypic variant (SeBac72) were generated and both were shown to retain oral infectivity in larvae. Interestingly, SeBac72 displayed a much higher transfection efficiency and viral spread in *S. exigua* Se301 and SeUCR cells as compared to SeBac10. We hypothesized that one or more gene(s) in the SeBac72 deleted region prevents successful virus replication of SeBac10 in cell culture. Roche 454 sequencing of SeBac72 revealed a 9.5 kb deletion spanning ORF16-28. Bacmids with different ORF16-28 knockouts were constructed to assess replication efficiency in Se301 cells. Se28 was identified as the responsible gene preventing successful replication of complete SeMNPV in cell culture. Transient expression of Se28 did not show any cytotoxic effect to Se301 cells. Expression of Se28 from a heterologous AcMNPV bacmid did not prevent virus replication in cell culture, indicating that the block on replication is virus-specific. Upon serial passage of bacmids expressing GFP from the polyhedrin promoter in Se301 cells, SeBac10 lost GFP expression after the 2nd passage, whereas GFP expression from SeBac72 and SeBac10ΔSe28 remained stable for at least 6 passages. Overall, the results identify Se28 as a key regulator of virus replication and show that deletion of Se28 leads to SeMNPV genome stability with retained very late gene expression in cell culture.

Contributed papers. Thursday, 08:45. **164 STU**

An ODV-specific baculovirus core gene *ha72* is essential for BV production and ODV occlusion

Huachao Huang[1], Manli Wang[1], Fei Deng[1], Basil M. Arif[2], Hualin Wang[1], Zhihong Hu[1]*
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Baculoviruses share a set of 37 core genes important for the virus life cycle. ORF72 of the *Helicoverpa armigera* NPV (*ha72*, a homologue of *ac78*) is a recently identified core gene with a hitherto unknown role in virus replication. It was identified as a late gene by time-course analyses of transcription and expression, and its encoded protein appears to localize to the intranuclear ring zone at the late stage of virus infection. Bioinformatics analyses revealed that HA72 contains a highly conserved IPLKL motif at the N-terminus and a fumarate reductase flavoprotein C-term, which putatively functions in redox reactions. Cells transfected with *ha72*-inactivated bacmid (bHaBacΔ*72-ph*) failed to produce infectious BV progeny, indicating its essentiality for BV production. However, it had no influence on viral DNA replication. Electron microscopy of bHaBacΔ*72-ph* transfected cells revealed irregular intranuclear membrane vesicles derived from the nuclear membrane suggesting HA72 may be involved in the production of membrane vesicles. Point mutations in the IPLKL motif implied a significant function for the amino acid lysine 22. A mutant, K22E curtailed BV production and precluded ODV occlusion. HA72 also interacted with P33; the latter being implicated in redox functions necessary for morphogenesis. It is probable that the two proteins form a redox complex during infection. These results demonstrated HA72 to be multifunctional. It has essential roles in the production of infectious BVs and occlusion of ODVs, and suggests a potential function in redox reactions through interaction with P33.

Contributed papers. Thursday, 09:00. **165 STU**

Mapping of the baculovirus AcMNPV ME53 residues essential for its nuclear translocation

Yang Liu, Eva Nagy and Peter Krell
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AcMNPV *me53*, a highly conserved immediate early gene, is found in all sequenced lepidopteran baculoviruses. ME53 contains a C4 zinc finger domain at the C-terminus, whose function is not yet clear. It translocates to the nucleus and colocalizes at the cell membrane with viral envelope protein GP64 in the late phase during infection. However, what mechanism ME53 uses to transport to the nucleus and whether ME53 interacts with other viral or host proteins to facilitate this translocation are still unknown. To determine which region is required for ME53 nuclear localization, HA-tagged ME53 truncations were constructed and immunofluorescence assays were performed. ME53 AA (83-152) was able to translocate into the nucleus in the late phase, while ME53 with AA (83-152) deleted failed to localize in the nucleus, indicating that residues within ME53 AA (83-152) is required for the nuclear translocation. GFP-fused ME53truncations were constructed to further narrow down the residues that are essential for the nuclear transport. When AA (1-106) was truncated, without the first 106 amino acids, the ME53 AA (107-449) nuclear transport was not inhibited. Interestingly, however, when AA (107-121) were further deleted, the rest of the peptide AA (122-449) mostly retained in the cytoplasm or in some cases, instead of translocating to the centre of the nucleus, it only accumulated along the nuclear membrane, and the nuclear transport was greatly abolished. This suggests that amino acid 107-121 in ME53 is essential for its nuclear translocation, which may also be relavant to the BV production and virus egress.

Contributed papers. Thursday, 09:15. **166 STU**

Temporal transcriptional analysis of *Cydia pomonella* granulovirus in the midgut of codling moth by using microarray analysis

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The *Cydia pomonella* granulovirus (CpGV) is the most widespread commercially used baculovirus and a cornerstone in the control of codling moth, *C. pomonella* L,. in both organic and integrated pome fruit production. Recently, codling moth populations resistant to CpGV products have been located in Europe. However, only limited information on the infection process of CpGV is available. To gain a better understanding of the interaction between CpGV and its host a microarray analysis of the transcription of CpGV genes in the midgut of codling moth was performed So far, on transcriptional level, there have been microarray analyses of infected cell lines with Group I and II Alphabaculoviruses only. First, an oligonucleotide based, 15k microarray covering the complete genome of CpGV was developed. Then, codling moth larvae were infected with CpGV and RNA samples were taken from midguts between 0 and 120 h post infection. The obtained microarray data were also compared to reverse transcription quantitative PCR. Microarray analysis of the different time

points resulted in a detailed overview of the temporal chronology of the transcription of all 143 CpGV genes. Five representative gene clusters were identified by performing a k-means clustering. Thereby, it was also possible to group undescribed CpGV genes according to their transcriptional profile. First transcriptional signals were detected between 12 and 24 h followed by a transcription boost of CpGV genes at 48 h; highest transcription activity was detected at 96 h post infection. A delayed and limited transcriptional activity of CpGV was observed in midgut`s of codlings moth strains resistant to CpGV.

Contributed papers. Thursday, 09:30. **167 STU**

Genomic adaptation to different hosts - What makes better-adapted viruses?

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The interplay between ecological and genomic adaptation is at the heart of evolutionary processes. Nucleopolyhedroviruses (NPVs), enclosing several virions in occlusion bodies (OBs), evolve as groups of genomes adapting to particular ecological niches. Shifts to a new niche should be linked with adaptation and differentiation of genome populations. To study how ecological adaptation affects NPV genomes, we conducted experimental evolution by passaging a wild type AcMNPV population on several host species, each defining a different ecological niche. AcMNPV OBs allow the maintenance of high genetic variation, because they contain more than 100 virions each enclosing several nucleocapsides, each with a genome. We discovered by ultra-deep Illumina sequencing of the AcMNPV wild-type isolate that a high diversity is maintained with different levels of polymorphism. We found few high frequency mutations located in essential genes involved in replication, transcription and transmission. Surprisingly we also revealed low frequency SNPs at every single nucleotide position of the genome. This suggests that any potentially adaptive mutation is already present within the population before experimental evolution. We also found large deletions by re-analysing the sequencing data with a new method taking into account the distances between reads. For the experimental evolution, we used the wild type AcMNPV to initiate 40 viral lines, 10 in each of 4 host species. After 10 passages, we repeated the deep sequencing to discover which genomic changes accompanied ecological adaptation.

Contributed papers. Thursday, 09:45. **168 STU**

Characterization of novel sequence in the Infectious Myonecrosis Virus genome *in* Pacific White Shrimp, *Litopenaeus vannamei*

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The emergence of Infectious myonecrosis virus (IMNV) in cultured shrimp occurred in Indonesia in 2006, caused huge economic losses to the aquaculture industry in South East Asia. IMNV is a member of the *Totiviridae* family and is a non-enveloped, monosegmented, doubled-stranded RNA virus that is 40 nm in diameter with icosahedral symmetry. Infected shrimp are lethargic, with progressively more white muscle fibers going from the tail to abdominal muscle; associated mortality in ponds can reach 70%. To explore host-pathogen interactions, we undertook a next generation sequencing approach (NGS) using lymphoid organ (LO) tissue from IMNV-infected shrimp. We reasoned that the LO would be enriched for immunity-related genes so would reveal valuable sequence information about the immune response to IMNV infection, and about how the virus genome is processed in infected shrimp cells. Surprisingly, our deep sequencing results showed that there were at least an additional 639 nucleotides at the 5' terminus and 23 nucleotides at the 3' terminus as compared to the original description of the genome (7560 nucleotides). The novel sequences were confirmed by RT-PCR and Northern analysis. Bioassays that employed double-stranded RNA to suppress this region of the genome revealed the critical nature of the new sequence to produce high titer infection and associated disease.

CONTRIBUTED PAPERS
Thursday 08:00-09:00

Fungi 4

Contributed paper. Thursday, 08:00. **169**

Deciphering the entomophthoralean genus *Tarichium*

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The form genus *Tarichium* within the Order Entomophthorales includes 39 species, the majority of which are rarely collected and are known only from Europe and from mites as hosts. Most species of Entomophthorales are known to produce two types of spores, conidia and resting spores, and historically, evolutionary relationships and the classifications of these fungi have been based on characteristics associated with conidia. Species of *Tarichium* are placed in this genus because they are only known to produce resting spores so no evolutionary relationships have been inferred about species in this genus. Although molecular taxonomic methods that are now available could help to provide evolutionary information, too few comparatively fresh collections of these taxa have been available for study. We report on an undescribed species of *Tarichium* from crane flies in New York State whose resting spores have a dark, roughened epispore–or (a)zygosporangial wall layer–that is not tightly attached to the internal, colorless, smooth (a)zygospore. DNA was extracted after breaking resting spores and degenerate primers were used to show that this species is most closely related to the genus *Zoophthora*. We will present hypotheses about the biologies and evolutionary relationships of this species as well as other species in this unresolved genus.

Contributed paper. Thursday, 08:15. **170**

The *Beauveria bassiana creA* is more than just a carbon catabolite repressor- role in nutrient toxicity, cellular development, and pathogenesis

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Carbon catabolite repression canonically involves the preferential utilization of glucose from a mixture of carbon sources and is found in the majority of both prokaryotic and eukaryotic organisms. The *creA* gene is a zinc-finger, DNA binding-protein homologous to the yeast *Mig1* catabolite repressor. Previous studies using RNAi directed gene knockdown of the *B. bassiana creA* homolog did not result in any significant phenotypes with respect to fungal development and virulence. Here, we report on the construction of a null mutant allele and show that compared with wild type and complemented strains, *B. bassiana* □*creA* displays a wide range of pleiotropic phenotypes. Fungal growth in media containing peptone or casein was severely compromised, with hyphal degeneration and autolysis occurring, a phenotype exacerbated at higher temperatures (32°C). Growth on rich and minimal media supplemented with various carbohydrates was also reduced in the mutant strain, and carbon source dependent pleiotropic

effects were seen regarding production of aerial mycelia and sporulation. Loss of *BbcreA* resulted in earlier sporulation and de-repression of protease and lipase activities. The development and production of blastospores, specialized single-cells produced by the fungus, was compromised in the □*BbcreA* strain. Insect bioassays indicated severe defects in virulence using both topical and intrahemocoel injection assays. In addition, under either infection protocol, eruption and subsequent sporulation on host cadavers was greatly reduced in the mutant. These data indicate *BbcreA* functions more than simply as a catabolite repressor and plays important roles in nutrient utilization, cell homeostasis, and virulence.

Contributed paper. Thursday, 08:30. **171 STU**

Group VIII histidine kinase in *Beauveria bassiana* is essential for the fungal growth, conidiation and adaptation to environment

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Filamentous fungi harbor a family of histidine kinases (HK) classified to 11 groups, of which most are functionally unknown and even considered as evolutionary remnants. Here we show for the first time that *Beauveria bassiana* Group VIII HK (BbHK8) with no paralog is functionally vital. *BbHK8* disruption mutant grew significantly slower on nutritional-rich media but much faster on several minimal media with different carbon/nitrogen source and availability. Perhaps due to a phytochrome domain in BbHK8, *ΔBbHK8* showed a rapid decrease of conidial yield at a rate of 5.7×10^7 conidia/h light under shorter light phase than 12:12 h light:dark cycle. The mutant was significantly more sensitive to two osmotic agents but more tolerant to two oxidants during colony growth, accompanied with partial losses of conidial thermotolerance and UV-B resistance. Surprisingly, the phosphorylation level of Hog1 hallmarking the high-osmolarity glycerol pathway of *ΔBbHK8* was greatly repressed under osmotic stress, supporting its high osmosensitivity. Our findings highlight the vital functions of BbHK8 in the nutritional uptake and adaptation to seasonal change and environment.

Contributed paper. Thursday, 08:45. **172 STU**

Cdc14 phosphatase acts as a nexus of cellular signaling network in *Beauveria bassiana* responses to developmental and stressful cues

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BbCdc14, a dual-specificity Cdc14 phosphatase located in the nuclei of entomopathogenic fungus *Beauveria bassiana*, was functionally characterized. BbCdc14 inactivation caused abnormal cytokinesis by forming multinucleate cells during hyphal development, slower growth on nutrition-rich and limited media and a loss of 96% conidial yield under normal conditions, accompanied with remarkable repression of 25 mitosis- and conidiation-related genes. Moreover, *ΔBbCdc14* became hypersensitive to oxidation, hyperosmolarity, carbendazim fungicide and cell wall disturbance during hyphal growth or conidial germination, and lost 41–70% of conidial thermotolerance, UV-B resistance and virulence. The reduced multi-stress tolerances were attributable to drastic repression of numerous stress-responsive genes, including most of mitogen-activated protein kinases (MAPKs) as well as Ras1/Ras2, PKA/ PKC, AMPK kinase Snf1, and enzymes or proteins associated with the fungal antioxidation, osmotolerance and cell wall integrity. The phosphorylation levels of Hog1 and Slt2, two hallmark MAPKs in *ΔBbCdc1*, were significantly reduced under all the chemical stresses. Thus, BbCdc14 regulates the fungal responses to developmental and stressful cues by acting as a nexus of cellular signaling network.

Nematodes 3

Contributed paper. Thursday, 08:00. **174 STU**

Evolution of virulence in an entomopathogenic nematode symbiont

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Photorhabdus is a genus of Gram-negative bacteria belonging to the Enterobacteriaceae. In addition to forming a mutualistic relationship with nematodes (Heterorhabditidae), these bacteria are primarily responsible for insect mortality during the nematode infection. There are three described species of *Photorhabdus*; *luminescens* and *temperata*, which are strictly entomopathogens, and *asymbiotica*, which has also been isolated from wound infections in humans. Phylogenetic relationships were investigated using parsimony and maximum likelihood analyses. Species formed strong monophyletic groups; however, subspecies placement was not as well-resolved. Prior to virulence assays, optimal growth conditions were investigated. Eight *Photorhabdus* strains with representatives from each species were grown in four media types; luria-bertani (LB) broth, LB+0.1% pyruvate (LBP), tryptic soy broth+0.5% yeast extract (TSY), and Grace's Insect Medium. All strains grew best in either LBP or TSY broths. However, when strains were plated onto agar plates the only medium on which all strains grew well was LBP agar. Therefore, subsequent assays were performed using LBP medium. To investigate how virulence has evolved in this genus, bacterial cells were injected into *Galleria mellonella* larvae, and the LT_{50} was calculated for each strain. These values were mapped onto the phylogeny using ancestral reconstruction methods. The results show that high virulence may have evolved multiple times in this genus and it might be strain dependent. Understanding how virulence has evolved in this bacterium will aid in unraveling the mechanisms of the *Heterorhabditis-Photorhabdus* complex, which may aid in the selection of nematode-bacterium complexes for biological control.

Contributed paper. Thursday, 08:15. **175**

Insect feed effect on entomopathogenic nematode development in the cadaver

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Entomopathogenic nematodes species *Heterorhabditis bacteriophora* infect and reproduce in wide range of insects, including herbivorous ones. In the present study we determined the effect of insect feed on the recovery and development of *H. bacteriophora* infective juveniles *in vitro* as well as *in vivo* Exposure of IJs to 70% ethanol extracts obtained from different dried leaves of local plant species including *Inula viscose*, *Pistasia lentiscus* and *Phillyrea latifolia* on artificial medium containing insect hemolymph. The extracts resulted in drastic reduction in recovery (60-100%) and resumption of the development. Similar effect recorded when last instars of *Spodoptera littotallis* were fed with Castor bean leaf discs dipped in crude solution of 70% ethanol extracts obtained from the above listed plants. The data indicate that these entomopathogenic nematodes may serve as a suitable model for investigation of feeding habits of host on the development of parasitic nematode/helminthes.

Contributed paper. Thursday, 08:30. **176**

Desiccation and heat tolerance of entomopathogenic nematodes-Transcriptome analysis

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Entomopathogenic nematodes (EPNs) in nature are exposed to extreme environmental stresses such as desiccation and heat. Substantial increase in survival at high temperature was achieved when EPNs were pre-exposed to high relative humidity or mild heat. An adaptation period is also needed for acquisition of the anhydrobiotic state, which allows survival under desiccation. We focus on the adaptation period of these two important stresses affecting nematode survival in *Steinernema* species, using whole transcriptome expression analysis of anhydrobiotic and heat stress related genes. For this purpose we used 454 pyrosequencing on three different nematode strains *Steinernema feltiae* strain IS-6 (SFG), *S. feltiae* Carmiel strain (SFCar) *S. riobrave* (SR). The 454 sequencing run obtained 26 to 50 million sequences per sample, 67,000-123,000 passed filter, averaging 374±12.6 bp. There were ca. 370,000 reads that were used for the assembly in all samples. We obtained 9274 uniqe transcripts that were functionally classified using Gene Ontology (GO) hierarchy. Transcripts have shown the best similarity (BLAST top-hit score) to the parasitic nematodes *Loa loa* (23.6%) and *Brugia malayi* (20.5%), *Caenorhabditis elegans* (14.2%), *Caenorhabditis briggsae* (12.6%), *Caenorhabditis remanei* (11.6%) and other non- nematodes species (17.4%). Analyzing gene expression patterns revealed an inverse correlation between gene expression and the phenotype of desiccation tolerance. SFCar was the most susceptible to desiccation and heat and had higher percentage of upregulated genes, while the stress-tolerance SR and SFG had higher percentage of downregulated genes. At the moment bioinformatics on the gene expression is still in process, and further analysis will be presented in the future.

Contributed paper. Thursday, 08:45. **177 STU**

New associations between *Deladenus* nematodes, their *Sirex* hosts, and fungal symbionts
E. Erin Morris[1], Ryan Kepler[1], Stefan Long[1], Elliott Zieman[2], David Williams[3] and Ann E. Hajek[1]
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The parasitic nematode *Deladenus siricidicola* is a biological control agent of the invasive woodwasp, *Sirex noctilio*. Since the discovery of established *S. noctilio* in northeastern North America in 2005, a biological control program involving *D. siricidicola* has been under consideration. We assessed native *Deladenus* spp. in the northeastern United States to predict possible non-target effects if *D. siricidicola* is introduced for *S. noctilio* control. Phylogenetic analyses were conducted on nematodes parasitizing *Sirex* spp. in the northeastern United States by collecting *Sirex* spp. in areas inside and outside of the range of *S. noctilio*. DNA was extracted from the nematodes, and two genes (CO1 mitochondrial DNA and ITS ribosomal DNA) were sequenced and analyzed. Results showed each species of *Sirex* to have a corresponding nematode parasite. Within two *Sirex noctilio* we found *D. proximus* nematodes which were normally associated with *S. nigricornis*. One of the native *Sirex nigricornis* contained *Deladenus siricidicola* which were normally found in *S. noctilio*. We provide evidence that *D. proximus* reproduces on *Amylostereum areolatum*, a fungus previously thought to be a non-host. We discuss nematode-host fidelity in this system and the potential for non-target impacts of a biological control program using *D. siricidicola* against *S. noctilio*.

Contributed paper. Thursday, 09:00. **178**

Feltiae/kraussei group of entomopathogenic nematodes: high intraspecific molecular diversity, or a presence of cryptic species?
Vladimír Půža, Jiří Nermuť, Martina Žurovcová, Lucie Faktorová, Daniela Chundelová, Zdeněk Mráček
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Increased sampling effort together with new molecular techniques in the last decade enabled a massive boom in the taxonomy and systematics of entomopathogenic nematodes (EPNs). In both Steinernematidae and Heterorhabditidae families, the number of species doubled from the year 2004 to the present 81 and 18 species, respectively. In general, molecular techniques facilitate characterizing and distinguishing of new nematode strains even where the traditional morphology-based system was insufficient. On the other hand, sometimes it may be difficult to distinguish between inter- and intra-specific variability. In present work, we focused on the strains from feltiae/kraussei group that were recently isolated from the territory of Czech Republic. Whole ITS region and D2D3 region of 28S rDNA were sequenced and the sequences were compared with feltiae/kraussei nematodes from laboratory collection that originate from different parts of the world and with sequences from Genbank. The analyses showed quite high molecular variability within established species with some genotypes recovered from all over the world, suggesting the existence of more independent lineages within some species. This fact has some important implications for EPN taxonomy.

Contributed paper. Thursday, 09:15. **179 STU**

Can fire gel improve entomopathogenic nematode application?
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A series of assays were conducted to assess the effects of Barricade© gel on entomopathogenic nematode (EPN) performance on leaf surfaces. These experiments are part of a larger body of work. The encompassing objective of the larger project is to improve the use of EPNs in an integrated approach to greenhouse pest control utilizing: strengthening plant defenses with silicon, insect mating disruption, and the use of EPNs to regulate herbivore populations. The main objectives of the assays at hand were to determine the optimum gel solution concentration to 1: improve nematode survival and 2: improve nematode host seeking. *S. carpocapsae* and *S. feltiae* were added to solution with the following gel concentrations: 0.25%, 0.5%, 1.0%, and 1.5%. EPN groups were applied via handheld sprayer to leaf surfaces in a greenhouse. EPN survival and movement was assessed at 0, 4, and 8 hours. At 0 hours 0.25% solution had the greatest proportion of living, active nematodes present, about 75%. At 4 hours, 1% has highest survival, and at 8 hours, survivorship was low for all groups, approx. 10%. The results demonstrated that 0.25% solution provides ideal conditions for nematode movement. Nematodes in 1.0% and 1.5% may be better protected against desiccation, but their movement is constricted by the denser formulation. This provides the foundation for further work integrating this application technique into practical pest management programs.

SYMPOSIUM (Cross Divisional)
Thursday, 14:00-16:00

Ecology of entomopathogenic co-infections

Symposium. Thursday, 14:00. **180**

Exploiting entomopathogen co-infections for biological control: current status and future directions
Helen Hesketh[1], Judith K. Pell[2,3] and Rosemary S. Hails[1]
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Insects are susceptible to infection by many pathogen species and mixed/concurrent infections are likely to be common in nature. The

outcomes of interactions between co-infecting pathogens are highly complex, ranging from independent to synergistic or antagonistic. One approach to improve biological control is to exploit the additive and synergistic interactions between pathogens and/or their toxins. For example, this may be through co-application of microbial products or seasonal use of functionally diverse groups of entomopathogens to control complexes of pests. For this to be successful we need to understand 1) the factors that influence positive (additive and synergistic) interactions between entomopathogenic micro-organisms and their hosts, 2) the mechanisms that may be sustaining these interactions and 3) how such interactions impact on the ecology of both the host insects and the infecting entomopathogens. Here, we review recent empirical evidence of interactions between entomopathogenic micro-organisms and nematodes specifically in lepidopteran and coleopteran larvae (both pest and non-pest species). We explore opportunities to exploit positive interactions to improve the efficacy of entomopathogens through increased host mortality and the extent of the evidence in the literature for this. In looking forward, we identify where research may be directed at knowledge gaps in understanding the mechanisms behind co-infection interactions. We also highlight the need for new methods of analysis to describe complex response patterns that incorporate dose-level or dose-ratio dependent interactions and present an example from mixture dose-response analysis of a *Bacillus thuringiensis*-nucleopolyhedrovirus interaction. This review will aid in further exploitation of entomopathogens for biological control.

Symposium. Thursday, 14:30. **181**

Interactions between fungi in *Plutella xylostella* larvae - which parameters have the greatest influence on the outcome of dual inoculations?

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Mixed infections have received increasing attention and it is generally accepted that they occur commonly in nature. Experimental evidence suggests that multiple infections may drive the evolution of virulence, but can also modify the survival rate and distribution of pathogens. We have studied interactions between the entomopathogenic fungi *Zoophthora radicans* and *Pandora blunckii* in *Plutella xylostella* populations. Both pathogens can co-exist in the same larval population. However, a number of different factors can affect their distribution. In dual-inoculation experiments, we found that both pathogens could infect and persist in larvae, either infecting fewer larvae than when inoculated alone, or through dual-infection. The latter was more likely when the *Z. radicans* isolate used was capable of producing resting spores. Both species could invade larvae that had already been inoculated with the other species, even when the first species had a time advantage of up to 12 hours. The highest proportion of dual-infected larvae occurred when the time lag between the first and second species was between 0 and 4 hours. Interestingly, the order of inoculation was more important than the time lag between the first and second species inoculated, in determining which species would ultimately be more successful in infecting *P. xyllostela* larvae. We found that the second pathogen inoculated resulted in the greatest number of infected larvae. We believe the larva´s immune system played an important role in determining which species was successful and are currently assessing the immune response of *P. xylostella* during staggered dual inoculations.

Symposium. Thursday, 15:00. **182**

Bacterial-fungal interactions in *C. elegans*

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In nature, microorganisms exist within polymicrobial communities, which abound with complex multispecies dynamics. Microbial survival is based on diverse bacterial-bacterial, fungal-fungal, and bacterial-fungal interactions. These ecological interactions in general and prokaryote–eukaryote interactions in particular, are likely important for the evolution and maintenance of microbial virulence toward humans. These interactions are ubiquitous in nature, as well as in clinical environments, but very little is known about the genetic mechanism(s) associated with these interactions. The soil-dwelling nematode *Caenorhabditis elegans* can be used to study the interactions between the prokaryotic gram-negative bacteria *Salmonella enterica* serovar Typhimurium and *Acinetobacter baumannii*, and the fungus *Candida albicans*. Both bacteria inhibit *C. albicans* filamentation, a key virulence determinant of *C. albicans*. This antagonistic, cross-kingdom interaction led to attenuated virulence of *C. albicans*, as determined by improved nematode survival when infected with both pathogens. The antagonistic interaction was also observed in a *C. albicans* biofilm environment. The *C. elegans*– *S.* Typhimurium–*C. albicans* interaction is mediated by *sopB*, an effector of a type III secretion system (TTSS) of *S.* Typhimurium. Deleting the *sopB* gene (which encodes inositol phosphatase) was associated with a significant decrease in *C. albicans* killing. SopB translocated to fungal filaments through SipB during coinfection. Interestingly, in the invertebrate host, the *sopB* effector negatively regulated the transcription of *CDC42*, which is involved in fungal viability. Interestingly, a likely evolutionary defense by *C. albicans* against *A. baumannii* exists, whereby *C. albicans* inhibits *A. baumannii* growth once a quorum develops. This counter-offensive is at least partly mediated by the *C. albicans* quorum-sensing molecule farnesol.
In summary, fungal–bacterial interactions are ubiquitous and have important medical and environmental significance. Within invertebrates, the interactions between these taxonomically diverse microorganisms are highly dynamic and dependent on a multitude of microorganism and host factors. Identifying the molecular mechanisms of this interaction in invertebrates may provide important insights into microbial pathogenesis.

Symposium. Thursday, 15:30. **183**

The evolution of virulence with mixed intra- and inter-specific infections in honey bees

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Honey bees are known to have a diverse pathogen community that can infect bees at different life stages. Some of the milder diseases caused by fungal pathogens have recently attracted more attention, especially in light of the multiple pathogen infections that can lead to higher host mortality. We used *in vitro* honey bee rearing to expose larvae to different fungal pathogens under strict laboratory conditions. Larvae were inoculated with several single strain and multiple strain co-infections of *Ascosphaera apis*, a specialist pathogen and causative agent of chalkbrood. In addition, larvae were inoculated with an opportunistic pathogen *Aspergillus flavus*, the causative agent of stonebrood disease as a single pathogen and also as a co-infection with *A. apis.* Our results show that the inter-specific co-infections of *A. apis* resulted in a lower host mortality compared to the single infections. Furthermore the intra-specific infections with *A. flavus* resulted in higher host mortality that followed the pathogen of more virulent pathogen, in this case *A. flavus*. This study demonstrates the unpredictable outcome of co-infections and highlights the importance of investigating these interactions that are commonly found in nature.

CONTRIBUTED PAPERS
Thursday, 14:00-15:15

Microbial control 3

Contributed paper. Thursday, 14:00. **184**

Dip treatment using *Isaria fumosorosea*: a potential biopesticide for mitigating the spread of invasive insects on ornamental plants pre- and post-shipping

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The efficacy of *Isaria fumosorosea* on the leaf phylloplane over time for controlling Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), nymphs before shipping plant products was assessed under laboratory conditions. Hibiscus leaves were dipped into beakers filled with 0, 1, 2, 4, 8, and 10 g of *PFR* 97® / L of water and tapped on brown paper towels to allow excess suspension to run off. Damp leaves were individually placed into empty plastic Petri dishes and a single 3rd - 4th-instar mealybug was exposed on the leaf. Each dish bioassay was sealed and placed in an environmentally controlled chamber at 25 °C under a 14 h L: 10 h D photoperiod. Percent mortality was determined after observing nymphs daily for 8 days post - exposure. The LT_{50} of nymphs after exposure to these dilutions varied from 6.9 – 11.1 days. Mortality of the mealybugs for concentrations 2, 4, 8, and 10 g / L was > 50% after 7 days post - exposure to PFR 97, but increased to 100% after final molting to the adult stage. The results suggest that molting may remove infective spores from penetrating the exoskeleton and subsequently decrease efficacy of the fungal treatments until the adult stage. Post-shipping studies using whole plants are in progress and results will be discussed. There is potential for using *I. fumosorosea* as dip treatment for mitigating the spread of the invasive insects (Madeira mealybug) on ornamental plants pre- and post-shipping.

Contributed paper. Thursday, 14:15. **186**

Integrated use of soil-dwelling predators and microbial biocontrol agents: Compatibility and efficacy against soil-dwelling stages of western flower thrips *Frankliniella occidentalis*

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Most western flower thrips (WFT) pupate in the soil. This laboratory study was designed to examine the compatibility and efficacy of soil-dwelling predators and microbial agents against pupating WFT, with a view to their concurrent use in an IPM strategy. All biocontrol agents used are commercially available. The soil predators tested were: a rove beetle, *Dalotia coriaria*, and two species of mites, *Stratiolaelaps scimitus* and *Geolaelaps gillespiei*. Two types of microbial biocontrol agents were used: entomopathogenic fungi, i.e. Met52® G (*Metarhizium anisopliae* strain F52) and BotaniGard® 22WP (*Beauveria bassiana* strain GHA); and nematodes, i.e. Nemasys® (*Steinernema feltiae*). According to IOBC pesticide selectivity criteria, Met52 and BotaniGard may be considered 'harmless' to 'moderately harmful' against the predatory species tested, causing mortality ranging from 2.93 % to 60.95 %. The mycoinsecticides were only moderately harmful to *D. coriaria* at the highest rates tested. All of the fungal and nematode treatments were harmless to *S. scimitus*. In contrast, all of the fungal and nematode treatments were moderately harmful to *G. gillespiei*. Efficacy against WFT significantly improved when the predators and fungi were applied together. This was not observed when the predators were used with nematodes, however, and no added benefits were derived from this combination. This may have resulted from predator feeding on the nematodes, with subsequent impact on efficacy against WFT, or access to a ready food source may have reduced thrips predation. Data from a recently-completed greenhouse study on use of combination treatments will also be presented.

Contributed paper. Thursday, 14:30. **187**

Progress in the microbial control of strawberry and vegetable pests: Research and extension efforts in California Central Coast

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Strawberry, leafy greens, and coles are major crops grown in the Central Coast region of California. Except for the use of *Bacillus thuringiensis* for controlling certain lepidopteran pests, microbial control is not commonly practiced in this region despite ideal environmental conditions for microbial agents such as *Beauveria bassiana*. Research has been conducted to evaluate the potential of commercially available formulations of *B. bassiana* alone and in combination with chemical and botanical pesticides for controlling strawberry and vegetable pests in various field studies. Efficacy of *B. bassiana* against cabbage and green peach aphids on broccoli, western flower thrips on lettuce, aphid, lygus bug, twospotted spider mite, whitefly, and thrips on strawberries was evaluated in commercial farms in large and small plot field trials. Compatibility of *B. bassiana* with some commonly used fungicides was also evaluated in laboratory assays. Results indicate that microbial control can play a major role in strawberry and vegetable IPM in California's Central Coast.

Contributed paper. Thursday, 14:45. **188**

Developing the particle size analysis technique to determine the hydrophobicity of fungal conidia and comparisons with two standard hydrophobicity determining methods

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Fungal conidia differ in hydrophobicity of their surface structures and this can cause different challenges when it comes to formulating mycopesticides. There are numerous methods to determine the hydrophobicity of fungal but, depending on the method used, different results can be obtained. The rank of hydrophobicity of a group of fungi may be an important factor as to which fungus is chosen to formulate into a product or how much surfactant may be required to obtain a homogenous suspension. A simple technique has been investigated to determine hydrophobicity by using laser diffraction. A particle size analyser (PSA) was used to determine the relative hydrophobicity of fungal conidia, Metarhizium sp. Trichoderma sp. Beauveria sp. and Alternaria sp., suspended in different liquids, Shellsol T, 0.05 % Tween 80 and distilled water. Not only does this method determine how easy it is for conidia to be suspended in a liquid but also how the conidia interact with each other in a particular suspension by measuring particle size i.e.clumps. To compare the results obtained from the PSA study, two other hydrophobicity tests, phase exclusion assay and salt-mediated aggregation and sedimentation (SAS), were performed on the same fungal samples. Of the three tests the PSA rankings were the most accurate when compared to microscopically looking at how the conidia suspend in different liquids. The SAS test was the next most reliable test, excluding Alternaria due to size differences. The phase exclusion test did not give reliable results for the Beauveria and Alternaria samples.

Contributed paper. Thursday, 15:00. **189**

Microbial products – New Zealand experiences

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A microbial product development programme has been funded by the New Zealand Government and industry for the last 5 years. The objective of the

programme was to develop novel products with potential for international markets, building on strengths from New Zealand experiences. During the programme, biopesticides, plant growth promotors and probiotics were investigated from a common base of microbial production, formulation and delivery science. Projects were selected for incorporation within a development pipeline with regular "stage gate" reviews for evaluation and reprioritisation. Bacteria, fungi and a virus were included as the active ingredients in the project mix. Our foray into probiotics delivered novel science but it has not been possible to transform this into successful commercial products. Greater success has been achieved among the biopesticides and biostimulants with two products arising from the research launched by industry and further potential products proceeding through the development pipeline. Spin-offs from the programme have included biopesticide development collaborations in Malaysia, Mexico, Ecuador and Uruguay. Views on the benefits and difficulties of applied research for product development will be presented.

CONTRIBUTED PAPERS
Thursday, 14:00-15:15

Viruses 5

Contributed paper. Thursday, 14:00. **190**

***Amsacta moorei* entomopoxvirus encodes a functional protein kinase**

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Amsacta moorei entomopoxvirus (AMEV) encodes a novel protein kinase gene (ORF AMV197), which is a homologue of poxvirus B1 protein kinase. Therefore, it is believed to play key role in virus replication. In this study, functional analysis of AMV197-null recombinant virus (AmΔPK/*gfp*) and substrate profile of protein kinase expressed in baculovirus vector system has been tested by RNA and peptide microarrays. Microarray analysis of viral gene transcription revealed significant differences between viral gene expression in AMEV and AmΔPK/*gfp* infected cells. Of all 617 tested substrates on peptide array, 80 were phosphorylated by expressed protein kinase. Taken collectively, the data indicate that the protein encoded by AMV197 may have significant effects on virus transcription and phosphorylation of wide range proteins. However, further investigations are needed to verify the exact role of this gene and effect on host range. **Keywords:** AmΔPK/gfp, *Amsacta moorei* Entomopoxvirus (AMEV), protein kinase, microarray **Acknowledgement:** This study has been funded by The Scientific and Technological Research Council of Turkey (Project No: 110T887) and Karadeniz Technical University, Scientific Research Fund (Project No: 8646).

Contributed paper. Thursday, 14:15. **191**

Analysis of the role of the *Lymantria dispar* enhancins on degradation of peritrophic membrane proteins from *L. dispar* larvae

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The *Lymantria dispar* M nucleopolyhedrovirus (LdMNPV) contains two *enhancin* genes that encode metalloproteases, which may enhance viral potency by degrading key peritrophic matrix (PM) proteins. Previous studies on the potency of recombinant viral constructs lacking one or both of the enhancin genes in the absence of an intact PM demonstrated that removal of the PM did not alter the relative potencies of these viral constructs. To investigate the role of LdMNPV enhancins on disruption of the PM, proteins from the PM were isolated and incubated with crude enhancin 1 (E1) protein extracts from polyhedra from wild-type, an E1 overexpressed recombinant (E1-OE), E2 overexpressed recombinant (E2OE), E1del, E2del, and E1delE2del viruses. Degradation of PM proteins was observed using polyhedra extracts from wild-type, E1del, E2 del, E1delE2del, E1OE, and E2OE viruses. Inclusion of the metalloprotease inhibitors EDTA, EGTA, phenanthroline, and Zn^{2+} did not inhibit PM protein degradation. Addition of a protease inhibitor cocktail containing AEBSF, aprotinin, bestatin, E-64, leupeptin, and pepstatin A (HALT) blocked degradation of PM proteins in the presence of E1OE polyhedra extracts. Addition of the serine protease inhibitor PMSF also blocked degradation of PM proteins when using E1OE polyhedral extracts. Together, these results suggest that the LdMNPV enhancin proteins are not degrading PM proteins within the context of the assay used for this study, and that the observed PM protein degradation was due to a serine protease.

Contributed paper. Thursday, 14:30. **192**

The banchine polydnavirus lineage: distinguishing features and evolutionary history

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Polydnaviruses (PDVs) are dsDNA viruses transmitted by endoparasitic wasps to caterpillar hosts during egg laying. In the caterpillar, PDV gene products induce physiological disturbances that enable the survival of the immature wasp. The encapsidated circular PDV genome segments are also present in linear form within the carrier wasp genome; this "provirus" serves as template for viral replication, which takes place exclusively in wasp ovaries. Strikingly, the encapsidated form of the virus is devoid of replication and structural protein genes, which are confined to the wasp genome. Characterization of wasp genomic regions harboring these genes has recently shed light on the distinct evolutionary origins of the two currently recognized PDV taxa, ichnoviruses (IVs) and bracoviruses (BVs), which are carried by ichneumonid and braconid wasps, respectively. While both IVs and BVs display comparable life cycles and genome structures, they derive from distinct ancestral viruses whose genomes integrated into the genomes of ancestors of the two wasp taxa. Most IVs characterized to date have been isolated from campoplegine ichneumonid wasps; however, PDVs are also found in banchine ichneumonid wasps, but these differ considerably from the former group with respect to gene content, degree of genome segmentation and virion morphology, raising the possibility of distinct evolutionary origins. To address this question, we used transcriptomics, proteomics and genomics approaches, focusing on genes that encode banchine IV virion proteins. The results point to the existence of homologs of campoplegine IV structural proteins in banchine IVs, suggesting a common or related ancestral viruses for the two lineages.

Contributed paper. Thursday, 14:45. **193**

Biocontrol of the box tree moth *Cydalima perspectalis*, an invasive pest in Europe, with *Anagrapha falcifera* nucleopolyhedrovirus (AnfaNPV)

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The invasive insect pest of many European countries, the box tree moth *Cydalima perspectalis* causes widespread damage on box tree plants. Investigations on the potential of *Anagrapha falcifera* nucleopolyhedrovirus (AnfaNPV) were conducted in this study. Two AnfaNPV isolates, termed Dn10 and BI-235, were used. AnfaNPV was identified to infect larvae of *C. perspectalis* by determining the partial nucleotide sequence of the three highly conserved genes *lef-8*, *lef-9* and *polh* of the infection causing agent. Additionally, light and transmission electron microscopic investigations verified high rates of infection in fat body, epidermis and tracheal matrix of *C. perspectalis* by both AnfaNPV isolates BI-235 and Dn10. The infectivity of AnfaNPV Dn10 and BI-235 to neonate larvae of *C. perspectalis* was evaluated by leaf disc bioassays. The median lethal concentration (LC_{50}) of both isolates were determined to 7.8×10^5 OBs/ml for isolate BI-235 and 2.3×10^6 OBs/ml for isolate Dn10 by using probit analysis. Thus, AnfaNPV BI-235 was significantly more virulent to neonate *C. perspectalis* larvae than Dn10 based on a three times higher LC_{50} value. In conclusion, the performed laboratory experiments indicate the susceptibility of *C. perspectalis* to AnfaNPV.

Contributed paper. Thursday, 15:00. **194**

Midgut transcriptomic response of the gypsy moth, *Lymantria dispar*, to infection with *L. dispar* and *Autographa californica* multiple nucleopolyhedroviruses

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Developmental resistance of gypsy moth (*Lymantria dispar*) larvae to baculovirus infection consists of both midgut-based and systemic components. To characterize the midgut response of larvae to baculovirus infection and identify larval host genes putatively involved in the midgut component of developmental resistance, we carried out a transcriptomic analysis of gene expression early after infection with *L. dispar* multiple nucleopolyhedrovirus (LdMNPV) or with a heterologous virus, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). Gypsy moth larvae at 48 h after the 3rd instar molt were allowed to feed on diet contaminated with a 10X LC_{99} quantity of LdMNPV-Ab-a624 OBs or an equivalent quantity of AcMNPV-C6 OBs, or with an equivalent volume of water, for 6 h. Larvae were then transferred to non-contaminated diet, and midguts were dissected 9 h after virus acquisition. Large-scale Illumina sequencing of cDNA prepared from midgut RNA was carried out. Assembly and analysis of the sequencing reads revealed that while many early LdMNPV genes were expressed in the midguts of infected larvae, virtually no AcMNPV gene expression was detected. Several host transcripts were elevated in LdMNPV-infected larvae relative to control (uninfected) larvae, including many transcripts with BLAST matches to putative 40S and 60S ribosomal protein genes. The impact of AcMNPV infection on host gene expression was much less pronounced. This study contributes to our understanding of host responses to baculovirus infection.

SYMPOSIUM
Friday, 09:00-09:30

NEMASYM

Symposium. Friday, 09:00. **195**

Myxococcal multicellular development as a defense against nematode predation

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Myxobacteria are gram-negative, soil-dwelling bacteria that are capable of two different coordinated lifestyles: cooperative-swarming predation of other bacteria and sporulation that is highly dependent upon the formation of multicellular fruiting bodies. Although these fruiting bodies contain hundreds of thousands of cells, only a fraction undergoes sporulation. Compared to their vegetatively-growing counterparts, myxospores are more resistant to stresses of heat, detergent, sonication, and enzymatic digestion. In addition to the two developmental states of swarmers and spores, *Myxococcus xanthus* is capable of reversible phenotypic switching ("phase variation") between yellow and tan variants. These two phase variants differ in abilities to swarm, survive in culture, and develop into fruiting bodies. However, it is not clear what fitness advantage either variant has in the soil environment. Recently myxobacteria was shown not to be at the top of soil food webs as these bacterial predators are themselves prey to *Caenorhabditis elegans*. *C. elegans* has a clear feeding preference for the tan variant and may play an ecological role in maintaining ratios of the two phase variants in the environment. However, *M. xanthus* is not defenseless against nematodes as the multicellular fruiting body acts as a steric hindrance to *C. elegans* feeding. After prolonged exposure to nematode predation, *M. xanthus* fruiting bodies become disseminated. Therefore, *C. elegans* appears to act both as a predator of *M. xanthus* and as a necessary agent of dispersal once *M. xanthus* has formed static fruiting bodies.

POSTER ABSTRACTS 2013

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STU indicates papers being judged for graduate student presentation awards

B-1 Loop 1 residues in domain II of Cry39Aa toxin are important for larvicidal activity against *Anopheles stephensi*.

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Cry39Aa from *B. thuringiensis aizawai* BUN1-14 is highly toxic to the mosquito larvae of *Anopheles stephensi*, which transmit malarial parasites. We developed a homology model of the Cry39Aa toxin using known structure of Cry4Ba toxin (PDB file 1W99) as template and identified predicted domain II loop 1, ^{349}KYAYWR^{354}. Many researches of Cry toxins have shown that loop regions of domain II are critically involved in toxicity and receptor recognition. To investigate functional role of loop 1 of Cry39Aa toxin, we performed site-directed mutagenesis in this region. In larvicidal activity, alanine substitutions revealed that the whole structure of loop 1, especially two aromatic amino acids Y^{350} and Y^{352}, is essential. However, Cry39Aa mutants with phenylalanine substitutions of two tyrosine residues (Y^{350}, Y^{352}) in loop 1 had no significant difference on toxicity as compared to wild-type Cry39Aa. Competition binding assay revealed that mutant toxins substituted to alanine showed reduced competition with wild type Cry39Aa toxin binding to *A. stephensi* brush border membrane vesicles (BBMV). These results suggest that the molecular structure of loop 1 in domain II of Cry39Aa toxin is important for toxicity and receptor binding of *A. stephensi*.

B-2 Characterization of a third promoter of the *cyt1Aa* gene of *Bacillus thuringiensis* subsp. *israelensis*.

Yuko Sakano,[1] Hyun-Woo Park,[1,2] Baoxue Ge,[1] Dennis K. Bideshi,[1,2] and Brian A. Federici [1,2]. [1] Department of Entomology, University of California, Riverside, Riverside, California, USA; [2]Department of Natural and Mathematical Sciences, California Baptist University, Riverside, California, USA; [3]Interdepartmental Graduate Program in Genetics, Genomics, and Bioinformatics, University of California, Riverside, Riverside, California, USA. (dbideshi@calbaptist.edu).

The virulence of *Bacillus thuringiensis* subsp. *israelensis* is due to synergistic interactions among four protein endotoxins assembled individually in a single parasporal body (PB) during sporulation. Cyt1Aa, the primary synergist, is the most abundant endotoxin, comprising approximately 55% of the PB's mass. The other proteins are Cry11Aa (35%), and Cry4Aa and Cry4Ba, which together account for the remaining 10%. The molecular genetic basis for the comparatively large amount of Cyt1Aa synthesized is unknown. Here, in addition to the known strong BtI (σ^E) and BtII (σ^K) promoters, we demonstrate a third promoter (BtIII), which has a high identity to the σ^E promoter of *Bacillus subtilis*, contributes to Cyt1Aa synthesis. Three transcripts were mapped in close proximity to BtIII. Moreover, we show that a BtIII-*cyt1*Aa construct was not functional in a σ^E-deficient strain of *B. subtilis*, and little or no Cyt1Aa was synthesized by the acrystalliferous 4Q7 strain of *B. thuringiensis* subsp. *israelensis* harboring site-specific mutations in the -35 and -10 boxes of BtIII. Comparative analyses of transcription levels and protein profiles of recombinant strains harboring different combinations of these promoters, or each alone, showed that BtIII is active throughout sporulation. From an applied perspective, as relatively low ratios of Cyt1Aa:Cry are known to synergize Cry proteins and to prevent or delay resistance to these in mosquito larvae, the results described here provide a foundation for genetically manipulating *cyt1Aa*'s *cis* elements to reduce the level of synthesis of this potent synergist, while maintaining its efficacy, with potential increases in Cry4Aa, Cry4Ba and Cry11Aa in *B. thuringiensis* subsp. *israelensis* strains being developed for use as mosquito larvicides.

B-3 Phylogenetic Distribution of Phenotypic Traits in *Bacillus thuringiensis* Determined by Multilocus Sequence Analysis

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Diverse isolates from a world-wide collection of *Bacillus thuringiensis* were classified based on phenotypic profiles resulting from six biochemical tests; production of amylase, lecithinase, urease, acid from sucrose and salicin, and the hydrolysis of esculin. Eighty two isolates representing 15 common phenotypic profiles were subjected to phylogenetic analysis by multilocus sequence typing; these were found to be distributed among 19 sequence types, 8 of which were novel. Approximately 70% of the isolates belonged to sequence types corresponding to the classical *B. thuringiensis* varieties *kurstaki* (20 isolates), *finitimus* (15 isolates), *morrisoni* (11 isolates) and *israelensis* (11 isolates). Generally, there was little apparent correlation between phenotypic traits and phylogenetic position, and phenotypic variation was often substantial within a sequence type. Isolates of the sequence type corresponding to *kurstaki* displayed the greatest apparent phenotypic variation with 6 of the 15 phenotypic profiles represented. Despite the phenotypic variation often observed within a given sequence type, certain phenotypes appeared highly correlated with particular sequence types. Our results suggest that the *B. thuringiensis* varieties *israelensis* and *kurstaki* represent the most abundant varieties in soil.

B-4 Can increased proteolysis be involved with *Helicoverpa zea* resistance towards Cry1Ac-activated toxin?

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A population of bollworm, *Helicoverpa zea* (Boddie) with over 200 fold resistance towards trypsin activated, purified Cry1Ac toxin has been maintained since 2005 and resistance has been demonstrated not to be binding based. Here we demonstrate increased trypsin- and chymotrypsin activity in midgut fluid from Cry1Ac-resistant *H. zea*, measured by enzyme-specific, chromogenic peptide substrates. Additionally, with ion exchange HPLC analysis we demonstrate how the extracted midgut fluids of resistant *H. zea* can further process trypsin-activated Cry1Ac at a rate greater than that for susceptible *H. zea* . These studies not only more closely define the role of trypsin- and chymotrypsin-like enzymes in the *H. zea* gut fluid regarding processing of activated Cry1Ac toxin, but they also suggest a role of increased proteolysis in Cry1Ac resistance by *H. zea*.

B-5 Insecticidal activity of Vip3Aa50 and Cry1Ea protein from *Bacillus thuringiensis* against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae

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The bacterium *Bacillus thuringiensis* is widely used to control agricultural pests due to entomopathogenic action and specificity of proteins. Among this proteins are Cry1 and Vip3 with activity in the control of lepidopteran pests. These toxins are highly specific and have no sequence homology between them. Bioassays were performed with proteins Vip3Aa50 and Cry1Ea expressed by *Escherichia coli* cells against neonate larvae of *Spodoptera frugiperda* with the objective to compare the efficacy in control of this polyphagous caterpillar. Protein lysates were applied on artificial diet in seven concentrations of each protoxin replicate four times and

each replicate with 16 larvae. Mortality was evaluated on the seventh day after exposure of larvae to the diet with protein. Lethal concentrations for 50% and 90% of larvae (LC_{50} and LC_{90}) were estimated by probit analysis using the statistical program POLO-PC (Leora Software. Berkeley, CA). The neonate larvae of *S. frugiperda* were more susceptibility to the Vip3Aa50 than Cry1Ea. The protoxin Vip3Aa50 showed a LC_{50} of 79.6 ng/cm^2 (51.1 - 129.6) and a LC_{90} of 547.5 ng/cm^2 (289.4 - 1630.8) with confidence limits of 95%. The protoxin Cry1Ea showed a LC_{50} ng/cm^2 3002.9 (2050.2 to 4904.3) and a LC_{90} of 16343.8 ng/cm^2 (8762.5 - 49643.9) with confidence limits of 95%. Proteins Vip3A has shown great potential in the application against insects not susceptible or resistant to Cry proteins.

B-6 Expression and toxicity analysis of *vip3Aa43* and *vip3Aa50* genes from *Bacillus thuringiensis* against *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) larvae.

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The Vip3A protein is an insecticidal protein secreted during vegetative growth phase of *B. thuringiensis*. It´s widely used in the control of lepidopteran pests through its expression in transgenic plants. The Vip3A proteins bind to specific receptors in the larva midgut membrane, which are different from those of Cry proteins, a fact which makes them an excellent alternative to the management of insect resistance to Cry proteins. Given this, the aim of this study was to obtain the isolation of novel *vip3A* genes and the toxicity analysis against larvae of *A. gemmatalis*. Thus, the genomic DNA of the strain *B. thuringiensis* var. *kurstaki* HD1 and the isolate I131 was obtained and amplified using primers based on the *vip3Aa1* gene sequence (gb [L48811.1]), from the amplified products were performed cloning these genes in the pET SUMO Champion™ (Life Technologies™) vector and the complete sequences of nucleotides were determined using primer walking strategy. The proteins expression was obtained using 0.4 mM IPTG for 5 hours at 22°C and the protein lysates were used in bioassays with *A. gemmatalis* neonate larvae. Both *vip3A* genes sequences showed 2370 bases and 789 amino acids. These sequences were submitted to GenBank databases and *Bacillus thuringiensis*: Toxin Nomenclature, and they were classified and grouped to the nomenclature of *vip3Aa43* gene (gb [HQ594534]) and *vip3Aa50* gene (gb [JQ946639]).The toxicity analysis of Vip3A proteins showed high efficiency for the control of *A. gemmatalis*, a fact which make them an excellent alternative to control and resistance management of this caterpillar.

B-7 Insecticidal activity of Vip3Aa, Vip3Ad, Vip3Ae, and Vip3Af from *Bacillus thuringiensis* against lepidopteran corn pests

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Cry proteins are the most well-known insecticidal proteins produced by *Bacillus thuringiensis* and have been extensively used in biological control as sprays and in transgenic plants (Bt-crops). Besides to Cry proteins, *B. thuringiensis* produces other type of insecticidal proteins, such as the Vip proteins (vegetative insecticidal proteins). Vip3Aa, Vip3Ad, Vip3Ae, and Vip3Af proteins (sharing around 82% identity at the amino acid level) were tested for their toxicity against *Spodoptera frugiperda* and *Agrotis ipsilon*, two important lepidopteran corn pests. Vip3Ad was non-toxic to the two species despite the high homology with the rest of Vip3A proteins. Vip3Ae and Vip3Af were significantly more toxic than Vip3Aa against *S. frugiperda*, both as protoxins and as toxins. Against *A. ipsilon*, Vip3Ae protoxin was more toxic than Vip3Aa and Vip3Af protoxins. Purification by metal-chelate affinity chromatography significantly affected Vip3Ae toxicity against the two insect species. Although we do not know if this extends to other Vip3 proteins, this possibility should be kept in mind when testing Vip3A proteins after purification by metal-chelate affinity chromatography.

B-8 Shared midgut binding sites for Cry1A.105, Cry1Aa, Cry1Ab, Cry1Ac and Cry1Fa proteins from *Bacillus thuringiensis* in two important corn pests, *Ostrinia nubilalis* and *Spodoptera frugiperda*

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First generation of insect-protected transgenic corn (Bt-corn) was based on the expression of Cry1Ab or Cry1Fa proteins. Currently, the trend is the combination of two or more genes expressing proteins with different modes of action. In addition to broadening the spectrum of action, this strategy helps to delay the evolution of resistance in exposed insect populations. One of such examples is the combination of Cry1A.105 with Cry1Fa and Cry2Ab to control *O. nubilalis* and *S. frugiperda*. Cry1A.105 is a chimeric protein with domains I and II and the C-terminal half of the protein from Cry1Ac, and domain III almost identical to Cry1Fa. The aim of the present study was to determine whether the chimeric Cry1A.105 has shared binding sites either with Cry1A proteins, with Cry1Fa, or with both, in *O. nubilalis* and in *S. frugiperda*. Brush-border membrane vesicles (BBMV) from last instar larval midguts were used in competition binding assays with ^{125}I-labeled Cry1A.105, Cry1Ab, and Cry1Fa, and unlabeled Cry1A.105, Cry1Aa, Cry1Ab, Cry1Ac, Cry1Fa, Cry2Ab and Cry2Ae. The results showed that Cry1A.105, Cry1Ab, Cry1Ac and Cry1Fa competed with high affinity for the same binding sites in both insect species. However, Cry2Ab and Cry2Ae did not compete for the binding sites of Cry1 proteins. Therefore, according to our results, the development of cross-resistance among Cry1Ab/Ac, Cry1A.105, and Cry1Fa proteins is possible in these two insect species if the alteration of shared binding sites occurs. Conversely, cross-resistance between these proteins and Cry2A proteins is very unlikely in such case.

B-9 Crystal-Forming Soil *Bacillus* from a Maryland Hardwood Forest are Predominantly Psychrotolerant Strains

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Crystal-forming *Bacillus* spp. were isolated from soil samples collected at different elevations within a mixed hardwood forest in central Maryland, and their phylogenetic relationships determined by multilocus sequence analysis. The vast majority of isolates obtained were associated with two phylogenetic groups known to be psychrotolerant, with very few isolates representing phylogenetic groups more typically associated with *Bacillus thuringiensis*. Isolates from these groups were confirmed to be psychrotolerant by growth on solid media at 7°C. Isolates of 11 highly related, novel sequence types from the psychrotolerant group that includes *Bacillus weihenstephanensis* were generally found at higher elevations, and were not associated with soils near streams. Isolates of 2 related sequence types from the second psychrotolerant group were nearly always found at the bottoms of ravines near streams.

B-10 Transcriptome of the gypsy moth (*Lymantria dispar*) larval midgut in response to Infection by *Bacillus thuringiensis*

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Transcriptomic profiles of the lepidopteran insect pest *Lymantria dispar* (gypsy moth) were characterized in the larval midgut in response to infection by the biopesticide *Bacillus thuringiensis kurstaki*. RNA-Seq approaches were used to define a set of 49,613 assembled transcript sequences, of which 838, 1,248 and 3,305 were respectively partitioned into high-, mid- and low-quality tiers on the basis of homology information. Digital gene expression profiles suggested genes differentially expressed at 24 hours post infection, and qRT-PCR analyses were performed for verification. The differentially expressed genes primarily associated with digestive function, including α-amylase, lipase and carboxypeptidase; immune response, including C-type lectin 4; developmental genes such as arylphorin; as well as a variety of binding proteins: cellular retinoic acid binding protein (lipid-binding), insulin-related peptide binding protein (protein-binding) and ovary C/EBPg transcription factor (nucleic acid-binding). This is the first study conducted to specifically investigate gypsy moth response to a bacterial infection challenge using large-scale sequencing technologies, and the results highlight important genes that could be involved in biopesticide resistance development or could serve as targets for biologically-based control mechanisms.

B-11 ***Anticarsia gemmatalis* and *Pseudoplusia includens* susceptibility to *Bacillus thuringiensis* Cry1 proteins.**

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The defoliating larvae of *Anticarsia gemmatalis* e *Pseudoplusia includens* are pests that cause great damage to agricultural crops, mainly soybean and cotton. An alternative for the control of these pests is the use of insecticidal proteins produced by *Bacillus thuringiensis*. Studies involving different Cry proteins can determine management strategies to delay or prevent the evolution of resistance for these insects through the production of pyramided transgenic plants involving toxins with different modes of action. Thus, the aim of this research was to evaluate the action of Cry1Aa, Cry1Ac and Cry1Ca proteins on *A. gemmatalis* and *P. includes* larvae, larvae growth inhibition and proteolitic kinetics using intestinal proteases of the larvae. The three Cry1 proteins tested were effective to control both pests. The Cry1Ac and Cry1Aa proteins were considered more effective on *A. gemmatalis* (LC_{50} 0.75 and 1.8 ng/cm^2) respectively while for *P. includes* Cry1Ca protein was the most effective (LC_{50} 7.7 ng/cm^2). Larvae growth inhibition reached 100% for *P. includes* when 30 ng/cm^2 were used for the three Cry toxins considered and 10 times lower for *A. gemmatalis*. Only 5% (v/v) of the larvae intestinal proteases were sufficient for complete activation of Cry1 proteins with 30 min of incubation, showing that there is no resistance in the population to activate these protoxins. The results of this study show the efficiency of Cry1Aa, Cry1Ac and Cry1Ca proteins tested in pest control and the importance of future studies in order to enhance the control of *A. gemmatalis* and *P. includens*.

B-12 **Discovery and Characterization of a Novel *Bacillus thuringiensis* Cry1B-Type Insecticidal Protein**

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Ostrinia nubilalis Hübner (European corn borer, ECB) is historically one of the most damaging pests of corn in the United States and Canada. Commercial corn varieties expressing insecticidal proteins Cry1Ab or Cry1F from *Bacillus thuringiensis* (Bt) have been in use since 1996 and 2003, respectively. Bt corn technologies to protect against ECB feeding damage have dramatically reduced commercial losses caused by this pest. Effective resistance management plans including the high-dose refuge strategy and pyramided proteins have resulted in trait durability despite the fact that individual populations resistant to Cry1Ab or Cry1F have been isolated in laboratory selection experiments and from the field. The potential for ECB to develop resistance necessitates the need to discover new Cry proteins with a mechanism of action different from Cry1Ab or Cry1Fa. Here we describe the activity spectrum of a novel Lepidopteran-active protein, Cry1Bh1, against several pests including Cry1F-resistant ECB. Cry1Bh1 is efficacious against both susceptible and Cry1Fa-resistant ECB in laboratory diet based assays. Cry1Bh1-expressing corn provided significantly better leaf protection against a Cry1F-resistant ECB strain compared to Cry1F-expressing hybrid corn. Further, Cry1Bh1 did not compete with Cry1Fa or Cry1Ab, for ECB midgut brush border membrane binding sites. These results suggest that the Cry1Bh1 mechanism of action in ECB is independent from Cry1Fa or Cry1Ab. Therefore, Cry1Bh1 can be considered a promising candidate to combine with Cry1Fa or Cry1Ab in transgenic corn to reduce the potential for ECB to develop resistance against these proteins.

B-13 **Receptor binding studies of a novel insecticidal protein, Arp095 with activity against a Lepidopteran pest**

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Transgenic crops expressing *Bacillius thuringiensis* proteins with insecticidal properties are demonstrating to be an effective tool for pest management. However, for the long term success of this technology, preemptive measures to combat pest resistance must be continually explored. One strategy for resistance management is to generate products containing two insecticidal toxins with different modes of action for each pest. We have used our diverse microbial strain collection to identify new toxins with activity against Lepidopteran pests. To determine if these novel toxins have unique modes of action, competitive receptor binding studies using brush border membrane vesicles from various Lepidopteran pests were conducted. Here we report the identification, purification and specific binding of Arp095, a novel insecticidal protein with activity against a key pest. We also demonstrate that the specific binding of Arp095 to gut receptors is not shared with Cry1 proteins, and therefore represents a novel mode of action against this pest.

B-14 ***Bacillus thuringiensis* Cry1Ca expressed in maize protects against feeding damage from susceptible and Cry1F-resistant *Spodoptera frugiperda***

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Bacillus thuringiensis insecticidal protein Cry1Fa is an important component of commercial varieties of insect resistant transgenic corn owing to its ability to protect against feeding damage by several economically important Lepidopteran pests including *Ostrinia nubilalis* (Hübner), *Diatraea grandiosella* (Dyar), *Spodoptera frugiperda* (J.E. Smith), *Agrotis ipsilon* (Hufnagel), *Striacosta*

albicosta (Smith) and several other stalk borers. *Spodoptera frugiperda* (FAW) field resistance to Cry1Fa corn was reported in Puerto Rico in 2006. Commercial cultivation of Cry1Fa corn in Puerto Rico was suspended in response to the occurrence of Cry1F-resistant FAW. This report describes the characterization of Cry1Ca as a candidate to prevent or delay development of Cry1Fa resistance in FAW when Cry1Ca and Cry1Fa are deployed as stacked traits in transgenic corn. We report here the biological activity and receptor competitive binding profiles for Cry1Fa and Cry1Ca. We demonstrated that Cry1Ca controls susceptible and Cry1Fa-resistant populations of FAW. We also show that Cry1Ca and Cry1Fa do not compete for binding sites in FAW midgut brush-border membrane vesicles. Furthermore, we demonstrated reduced feeding damage of FAW and Cry1Fa-resistant FAW on Cry1Ca corn. These data support the pyramiding of Cry1Ca and Cry1Fa to delay the development of resistance in FAW.

B-15 Use of a pooled clone method to isolate a novel *Bacillus thuringiensis* Cry2A toxin with activity against *Ostrinia furnacalis*

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A pooled clone method was developed to screen for *cry2A* genes. This metagenomic method avoids the need to analyse isolated *Bacillus thuringiensis* strains by performing gene specific PCR on plasmid-enriched DNA prepared from a pooled soil sample. Using this approach the novel holotype gene *cry2Ah1* was cloned and characterized. The toxin gene was over-expressed in *Escherichia coli* Rosetta (DE3) and the expressed toxin accumulated in both the soluble and insoluble fractions. The soluble Cry2Ah1 was found to have a weight loss activity against *Ostrinia furnacalis*, and a growth inhibitary activity to both Cry1Ac-sensitive and resistant *Helicoverpa armigera* populations.

B-16 Investigation of the spore concentration of *Bacillus thuringiensis* on marketable Salanova-lettuce after application of Xentari®

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In 2012 lettuce samples were found in the food trade with high *Bacillus thuringiensis*/ *Bacillus cereus* concentrations. Because of this indication we proofed which spore concentration can be achieved on Salanova-lettuce after a standardized application of Xentari® immediately before harvesting. For these experiments marketable heads of salad were transferred from the field to the lab. Afterwards, the heads were treated with following application adjustments: 2x200 l/ha, 3 bar, speed of 3.5km/h, Teejet 8003 EVS nozzle and an application rate of 1000g Xentari® per ha. These adjustments and application rates were in accordance to the application of the farmer. After application lettuce samples were taken and the number of colony forming units (cfu) per gram fresh weight was determined. In average concentrations of around $5x10^5$ cfu/g were deterimined after treatment with Xentari®. The values ranged between $5.4x10^4$ und $2.1x10^6$ cfu/g. In the untreated control values of up to $2x10^5$ cfu/g fresh weight were counted. The results illustrate that after an application of Xentari® a concentration of the B. *cereus* group can be higher than the benchmark of $1x10^5$ cfu/g.

B-17 STU Rapid isolation and characterization of a new/novel *Bacillus thuringiensis* strains from animal manure samples and their toxicity to the Coleoptera

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Bacillus thuringiensis (Berliner) (*Bt*) wonder insecticide has become the neatest successes among the microbial pesticides. The present research was focused on the isolation and characterization of *Bt* from animal manure samples. Initially, selection was done mainly based on colony morphology, Gram staining, phase contrast microscopy and scanning electron microscopy. Total of 96 colonies were identified and isolated based on colony morphology, in which off-white, matte and creamy appearance showed the characteristics of the genus *Bacillus*. The number reduced to 39 colonies after Gram staining was done. Furthermore putative native *Bt* strains were characterised for the presence of Coleopteran active *cry* gene content by PCR analysis, cloning, sequencing and Cry toxin content by SDS-PAGE profiling. PCR analysis revealed the presence of *cry7Ab* and *cry1* like genes followed by *cry1I, cry3C, cry3A* and *cry7, 8*. Subsequently all these genes were cloned sequenced and submitted to the NCBI GenBank. SDS-PAGE analysis revealed that these isolates produced two major ranges of polypeptides, the Cry protein which corresponds to a range of 66-140kDa. The evaluation of native *Bt* strains were done against *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae) through leaf dip bioassay. In conclusion given the variability of insecticidal proteins described to date, the isolation and characterization of various new subspecies of *B. thuringiensis* could contribute to the discovery of very effective biopesticide with higher insecticidal activities and broader host range against insect pests especially in the sectors of agriculture and forestry, and vectors of medically important diseases of human beings and other animals.

B-18 Isolation and characterization of *Photorhabdus temperata* (Proteobacteria: ENTEROBACTERIACEAE) from *Heterorhabditis safricana* in Costa Rica

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Photorhabdus is a genus of entomopathogenic Gram negative bacteria that maintains a mutualistic association with nematodes. In Costa Rica, only the species *P. luminiscens* and *P. asymbiotica* have been reported. Given the potential use of *Photorhabdus* sp in the production of antimicrobial agents, the aim of this work was the biochemical and molecular characterization of the bacterial strain CRCIAP-01, isolated from Heterorhabditis safricana, entomopathogenic nematode collected in Zarcero, Costa Rica. Further molecular characterization by amplification and sequencing of the genes gyrB and 16srRNA was conducted.

Phenotypic traits concord with the characteristics described for the genus *Photorhabdus*. The isolate does not reduce nitrate to nitrite, was dehydroxylase and decarboxylase negative, and fermented a very limited number of carbohydrates, was resistant to penicillin and has characteristics typical of the genus *Photorhabdus* as bioluminescence, pigmentation, protein inclusions, swarming phenomenon and the production of antimicrobial agents that inhibit the bacteria *M. luteus*, *E. coli* and *S. aureus* and fungi: *Fusarium*, *Rhizoctonia solani* and *Phytoptora capsici*. Through analysis of 16s rRNA sequences and gyrB, the bacterium was identified as *P. temperata* and the constructed phylogenetic trees show that this species is related to sequences of strains isolated from America GPS11, Havana, C1, OH1, and MEG1 WX6. This work represents the first isolation of the strain *P. temperata* in Costa Rica and the first time it is found associated to *Heterorhabditis safricana*.

B-19 Biochemical and molecular characterization of symbiotic bacteria of four Steinernema from Costa Rica: S. costaricense n.sp. (CR9), S. puntauvense n. sp. (Li6), S. websterii (CR5) and Steinernema sp. (T4) (Rhabditida: Steinernematidae)

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It´s estimated that 5% of the world biological richness is found in Costa Rica, whose protected areas encompassed 26% of the country. Surveys conducted in 4 protected areas found four Steinernema species. S. costaricense n. sp. (CR9), S. puntauvense n. sp. (Li6), S. websteri (CR5) and Steinernema sp (T4). Bacterial isolates extracted were characterized by biochemical, BIOLOG, API 20E, 20NE, and sequence analyses of the 16S rDNA gene. Similarity matrix were calculated and cluster analyses were performed by UPGMA method. The derived dendrogram based on phenotypic traits placed the four Costa Rican Xenorhabdus isolates into three different clades: CR5 (S. websterii symbiont) was positioned into one clade near to X. nematophila and the symbionts of S puntauvense and S. costaricense were placed into another clade, but belonging to two different clusters. S. puntauvense symbiont was more closely related to X. bovienii, and the Xenorhabdus sp. associated to S. costaricense was positioned in a separate cluster. Xenorhabdus sp. isolated from Steinernema sp. T4 was placed in a third clade alone. Sequence analyses of 16S rDNA genes confirmed Xenorhabdus CR5 have 97% identity with to X. nematophila (RIOBRAVIS), Xenorhabdus Li6 had a 95% of similarity with X. bovienii, CR9 shared 95% of similarity with X szentirmaii and the isolate T4 95% with a Xenorhadus sp. strain. Further analyses is required to find if the T4, CR9 and Li6 isolates represent new species of Xenorhabdus genera. This study contributes to the knowledge of the biodiversity of entomopathogenic bacteria in Costa Rica.

B-20 Molecular mechanism of the *Bacillus sphaericus* mosquitocidal plasmid pBsph partitioning

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Bacillus sphaericus plasmid pBsph encodes binary toxins (Bin toxins), which are toxic toward mosquito larvae. Although the aspects of Bin toxins have been studied extensively, much less is known about the control of pBsph replication and maintenance. Here, a 2.4-kb DNA fragment encoding ORF189 and a downstream gene (ORF188) was identified as the minimal replicon that is necessary and sufficient for pBsph replication and stability. Both ORF188 and ORF189 are homologs of TubZ and TubR, and designated here as TubZ-Bs and TubR-Bs, respectively. TubR-Bs bound specifically to eleven 12-bp degenerate repeats in three clusters, whereas the GTPase TubZ-Bs was recruited into TubR:DNA complex, leading to the formation of a type III partition complex. Electron microscopy (EM) showed that TubZ-Bs assembled long filaments in a GTP-dependent manner, and *in vivo* the TubZ-Bs-GFP filaments were highly helical and extended to the opposite poles of the dividing cell. A point mutation (Y260A) in TubZ-Bs that completely abolished the GTP hydrolysis and polymerization *in vitro* was strongly defective in replication or segregation. Another point mutation (T114A) that exhibited relatively lower GTP hydrolysis rate and polymerization activity dramatically decreased the plasmid copy number, which, however, could be largely complemented by a 1.3-kb DNA sequence downstream for ORF187. Further studies demonstrated that an upstream gene of TubR-Bs encoding ORF001 could promote TubZ-Bs assembly. Such new elements could deepen our understanding of the diversified modulation on the plasmid partition systems.

B-21 A collagen-like glycoprotein Bsph_0411 is required for the formation of filamentous structure of the internal exosporium of *Bacillus spaericus*

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Bacillus sphaericus is an aerobic, spore-forming and Gram-positive bacterium which could produce mosquitocidal binary toxins (Bin toxins). The crystal toxins are enveloped by the outmost loose-fitting and balloon-like exosporium that protects them from degradation and contributes to the persistence of these toxins in nature. Although the Bin toxins have been studied extensively, much less is known about the organization and composition of this exosporium. Bioinformatic analyses revealed that Bsph_0411, encoding a protein of 1,142 amino acid residues, showed 52% amino acid identity to BclA from *Bacillus cereus* and contained 327 triplet repeats including 117 GAT and 85 GVT that is typical of a collagen-like region. Results showed that Bsph_0411 was a glycoprotein and located exclusively on the surface of the exosporium as revealed by immunogold labelling. Thin-section electron microscopy revealed that the null mutation of Bsph_0411 resulted in the absence of the filamentous structure in the internal exosporium, indicating a main function of this protein involved in the formation of exosporium. In vivo visualization of the Bsph_0411-GFP fusion protein in *B. sphaericus* revealed a dynamic pattern of fluorescence that follows the formation sites of the exosporium around the forespore, and the fluorescence was mainly localized on the mother cell proximal pole of the mature spores. Our results demonstrated a collagen-like glycoprotein (Bsph_0411) that may play a role in maintenance the balloon-like structure of the *Bacillus sphaericus* exosporium. This study is of importance for further understanding the mechanism of the protection of the exosporium for the Bin toxins and spores.

B-22 STU *Xenorhabdus nematophila* suppression of *Manduca sexta* immune surveillance

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The *Xenorhabdus nematophila* transcription factor Lrp contributes to this bacterium's mutualistic relationship with nematodes, its pathogenesis and immune suppression in Lepidopteran insects, and to phenotypic variation. *lrp* null mutants are attenuated in virulence, defective in suppression of antimicrobial peptide expression and trigger hemocyte aggregation. Variation in Lrp protein levels contributes to virulence modulation, a phenomenon in which some bacterial cells in a clonal population display attenuated virulence while others retain a virulent phenotype. Low levels of Lrp are associated with a virulent and immuno-suppressive phenotype, while cells expressing high Lrp levels are attenuated and immuno-stimulatory. The specific aspects of immunity targeted by *X. nematophila* Lrp-regulated genes have not been elucidated. Using qRT-PCR we show that *X. nematophila* Lrp plays a role in suppressing the expression of hemolin, a Lepidopteran-specific pattern recognition receptor. An *X. nematophila lrp* mutant fails to suppress levels of hemolin transcript, indicating Lrp is necessary for suppressing surveillance mechanisms. Further experiments will address the role of hemolin in fighting infection by *X. nematophila* and the mechanism by which *X. nematophila* suppresses this activity.

B-23 STU Toxicity of five Cry1 proteins against *Diatraea saccharalis* (F, 1794) (Lepidoptera: Crambidae): synergism-antagonism analysis

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Some crops are being engineered with more than one *cry1* gene from *Bacillus thuringiensis* in order to protect them from several lepidopteran pests. So, it is important to evaluate possible synergistic or antagonistic interactions between these proteins. In the present study, a Brazilian population of *Diatraea saccharalis* has been tested for susceptibility to five Cry1 trypsin-activated proteins (Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca and Cry1Ea) by artificial diet surface contamination. At the level of LC_{50}, Cry1Ab was the most active protein (0.35 ng/cm^2) followed by Cry1Ac (5.1 ng/cm^2) and Cry1Ca (5.8 ng/cm^2) with a similar toxicity. Cry1Aa showed an LC_{50} around 43 ng/cm^2 and the less effective one was Cry1Ea, which showed no toxicity to this species. The LC_{50} values for Cry1 trypsin-activated toxins were lower than their respective LC_{50} values obtained for protoxin samples, suggesting that the activation step is rate limiting. We had previously shown that Cry1Aa, Cry1Ab and Cry1Ac share the same receptors in this pest and that Cry1Ca has a different one. For this reason we have chosen Cry1Ca and Cry1Ab (the most effective one) to study possible interactions affecting toxicity to *D. saccharalis* larvae when these two toxins are ingested in combination. We have found a slight antagonism between then, suggesting that these toxins should not be used on a pyramided transgenic crop.

B-24 STU Testing the role of arylphorin in midgut healing after Cry1Ac intoxication in *Heliothis virescens* larvae

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Upon pathogenic infection, the integrity of the lepidopteran larval midgut epithelium is maintained by tissue healing mechanisms that are mediated by stem cell proliferation and differentiation. This healing process is only active during molting or following injury to mature cells lining the lumen-exposed epithelial surface. A limited number of midgut growth factors have been identified from hemolymph and fat body tissue extracts, but the factors involved in regulation of the midgut healing response to pathogenic damage are not known. Our work is focused on the midgut healing response in *Heliothis virescens* (tobacco budworm) larvae elicited by challenge with the Cry1Ac toxin from the bacterium *Bacillus thuringiensis*. Through a differential proteomic analysis using primary midgut cell cultures, we have identified arylphorin as a significantly up-regulated protein in response to Cry1Ac intoxication. Arylphorin, a hexameric storage protein that is traditionally recognized to be synthesized and released from the fat body, is primarily known for its role during pupation and the formation of adult tissues. However, there is evidence that arylphorin is also synthesized in the midgut epithelium. In the current work we present data from experiments designed to test the functional role of arylphorin as a midgut healing factor upon Cry1Ac intoxication in *H. virescens* larvae. We report data from feeding bioassays and *in vitro* binding assays that support a crucial role for arylphorin in activating the insect midgut healing response. In addition, our data also support the relevance of midgut healing for insect susceptibility to Cry toxins.

B-25 STU Selection of proteins Cry1 active against the lesser cornstalk borer, *Elasmopalpus lignosellus*

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The use of biological control agents is an alternative to the intensive use of chemical insecticides in the management of insect pests because the latter cause reduction of natural enemies, rapid evolution of insect resistance, environmental pollution and high cost of production. The control of *Elasmopalpus lignosellus*, an important lepidopterous pest of several crops can be accomplished using Cry protein from *Bacillus thuringiensis*. Accordingly, the aim of this study was to evaluate the potential control of proteins Cry1Aa, Cry1Ac, and Cry1Ca in a Brazilian population of the pest. Recombinant *Escherichia coli* clones carrying genes were cultivated only in the medium for inducing proteins and lysates were used for toxicity tests using bioassays. Different protein concentrations placed over the diet were offered to neonate larvae of *E. lignosellus*. Mortality (%) was evaluated after seven days and the results were subjected to probit analysis to estimate the LC_{50}. The LC_{50} of toxins Cry1Aa, Cry1Ac, and Cry1Ca found for *E. lignosellus* were 73.63, 15.63, and 36.10 ng/cm^2, respectively. The LC_{50} for Cry1Ac was about five times lower than that obtained for Cry1Aa and it was twice as lower than Cry1Ca. Thus, this study showed that all three proteins Cry1 are effective in controlling *E. lignosellus*, which make them important sources of Bt proteins in the production of transgenic plants resistant to this insect pest.

B-26 Selection for Cry1Ie resistance in the Asian corn borer and cross-resistance to other Cry toxins

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Asian corn borer, *Ostrinia furnacalis* (Guenée), is one of the most important insect pests of maize in China. Transgenic *Bacillus thuringiensis* (Bt) maize provide an effective mean to control this insect pest. However, it will be the great threat to the continued success of Bt toxins used in insecticide formulations or expressed by transgenic maize for the evolution of resistance by the target insect. It is theoretically and practically important for well-characterized resistant strains, which will provide the only way to empirically validate proposed management strategies. A strain of *O. furnacalis* originated from field collection of Xi'an, Shanxi Province was selected for resistance to Cry1Ie by exposure to the toxin incorporated into artificial diet in the laboratory. The susceptibility of selected strain to Cry1Ie toxin was declined with the selection pressure increased. The selected strain developed more than 23-fold resistance to Cry1Ie after 14 generations of selection. However, it was as susceptible to Cry1Ab, Cry1Ac, and Cry1F as the unselected control strain. In addition, comparing with the unselected strain, the larvae of selected strain required an average of 5.7 d longer to develop; the pupal weight was reduced by 13.7%; and the number of eggs laid per female was decreased by 40.0%.

DB-1 Israeli acute paralysis virus in bumblebees: control by radiation and RNAi

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Israeli acute paralysis virus (IAPV) is lethal after injection in honeybees. It has also been reported in non-*Apis* hymenopteran pollinators such as bumblebee species, which can become infected when placed in the neighborhood of infected honeybee hives. We saw that injection of only 20 particles of IAPV resulted in bumblebee mortality within 7 days. Therefore we looked at different measures to prevent IAPV infection and bumblebee mortality. Eradication of IAPV is important, knowing that annually more than 1 million bumblebees colonies are sold for pollination purposes, and a virus-free status is desired for transport of bumblebees. By use of gamma radiation of IAPV (15kGy) we were able to inactivate viral particles. In parallel, we investigated the use of RNAi technology with dsRNA molecules targeting different viral open reading frames, to rescue infected bumblebees.

DB-2 ***Areospora rohanae*** **n.gn. n.sp. (Microsporidia) causes host cell syncitium formation in the Antarctic king crab (*Lithodes centolla*)**

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Antarctic king crabs (*Lithodes centolla*), a highly prized commercial fishery target in Chile, were sampled from processing plants during 2012. Crabs displaying characteristic lesions (white, opaque raised nodules within sub-cuticular tissues) were processed for histology, electron microscopy and molecular diagnostics. Macroscopically the lesions resembled xenomas caused by infection with microsporidian parasites. Histology revealed extensive remodelling of the sub-cuticular connective tissues. Presence of parasites within connective tissues of the walking limbs, gill lamellae, and hepatopancreatic haemal sinuses suggested a primary infection site within the fixed phagocyte clusters (FPC) of these tissues. Early infection of the FPC cytoplasm (by uninucleate stages) progressed to colonisation of the cytoplasm by multiple parasite stages. Characteristically, fusion of infected FPCs formed a distinctive syncitium (giant cell) in which multiple host nuclei could be observed predominantly at the periphery but also amongst parasite cells. Presumed fusion with adjacent infected syncitia initiated the progressive remodelling of the connective tissue matrix. Giant syncitia were separated by fibrous remnants of the connective tissue matrix. Crabs displaying clinical signs were shown to be infected with a novel microsporidian parasite. Ultrastructural observations of infected tissues revealed numerous merogonic and sporogonic life stages which culminated in the production of bizarre spores, ornamented with distinctive bristles emerging from the exosporal layer. Uninucleate mature spores occurred in sets of 8 within a sporophorous vesicle. Partial sequencing of the SSU rRNA gene revealed high divergence to extant taxa. Histological, ultrastructural, phylogenetic and host ecological data is utilised to propose the erection of *Areospora rohanae* n.gn., n.sp. within the phylum Microsporidia.

DB-3 **New block on the kids: highly-divergent *Mikrocytos*-like parasite infects juvenile crabs**

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We have recently discovered an apparently amitochondrite protistan pathogen infecting the bladder of the European edible crab (*Cancer pagurus*). The parasite appears to specifically infect juvenile life stages (up to 70% prevalence). Uninucleate stages of the microcell pathogen reside within the cytoplasm of bladder epithelial cells where they undergo nuclear fission to form multinucleate plasmodia. Plasmotomy appears to create further uninucleate life stages from these large plasmodia. Uninucleate and plasmodial stages are ejected into the bladder lumen for presumed excretion via the urine. No spore stages have been observed. Shotgun sequencing (Illumina MiSeq) of total DNA extract from infected bladder tissue resulted in a metagenomic dataset that was mined for small subunit ribosomal RNA genes (SSU rDNA) and other protein-coding genes known to be phylogenetically informative in pan-eukaryote analyses. All genes analysed were highly divergent relative to other eukaryotes which meant that a highly resolved phylogenetic position could not be calculated. However, the best support was attained for affinity with a *Mikrocytos*-like lineage branching within the Rhizaria. This was supported by core rhizarian (Cercozoa-Foraminifera) sequence signatures in the SSU rDNA and polyubiquitin genes. The crab parasite showed highest sequence similarity to the oyster microcell pathogens *Mikrocytos mackini* (HM563060) and three other *Mikrocytos*-like species. Although similarity was only 83%, the SSU rDNA trees showed that they were robustly related. The finding of a highly prevalent *Mikrocytos*-like pathogen infecting juvenile life stages of an important commercially exploited decapod is intriguing. The relative absence of the pathogen in adults contrasts findings of *M. mackini* infections in oysters (an adult pathogen). Furthermore, since the evolutionary history of *Mikrocytos* is almost completely unknown (being distant to any known protist), the high prevalence of a similar pathogen, and the relative ease in which it can be isolated from infected crabs, makes it an attractive model to study early eukaryotic evolution.

DB-4 **Differential *Nosema bombi* (Microsporidia: Nosemidae) incidence in colonies of *Bombus occidentalis* and *Bombus huntii* (Hymenoptera: Apidae)**

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Nosema bombi has been implicated in the decline of several species of bumble bees in North America, including the western bumble bee, *Bombus occidentalis.* Whereas wild populations of *B. occidentalis* have been demonstrated to harbor *N. bombi* infections with greater incidence and higher intensity than other North American bumble bee species, another common western bumble bee species, *Bombus huntii*, has been shown to have lower levels of *N. bombi* in the wild. Despite the decline of *B. occidentalis* and the current development *of B. huntii* as a commercial pollinator, little is known about the prevalence of this pathogen in laboratory reared colonies of these two species. We raised eight *B. occidentalis* colonies alongside eight *B. huntii* colonies in the laboratory from queens captured in the wild. Colonies were allowed to develop in captivity alongside each other in the laboratory, but individuals were not allowed to directly intermingle. We removed 25 individuals from each colony and removed the gut tissue for microscopic examination for *N. bombi*. *Bombus occidentalis* colonies had a higher infection level and a greater spore load than did *B. huntii*. Differential levels of *Nosema bombi* are found in colonies of the two species of bumble bees maintained in captivity. Implications for management and the health of wild populations are discussed.

DB-5 STU **Do weather conditions have an impact on the incidence of *Nosema* spp. in the European honey bee (*Apis mellifera*)? - A case study of bee colonies from North-east Germany**

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In the last decade, the wide spread loss of the European honey bee (*Apis mellifera*) colonies due to a variety of causes has been cause for concern as honey bees play an important role in the pollination of several commercially important crops and plant communities in the wild. Microsporidial infections (*Nosema apis* and *Nosema ceranae*) have been identified as one of the potential contributing elements, in particular to winter losses. *N. ceranae* is an emerging pathogen in *A. mellifera* and seems to be very competitive, therefore replacing *N. apis*.in some parts of the world. Identification of factors that influence the incidence of *Nosema* spp. is necessary to forecast the infection risk in the coming season and take adequate measures to both mitigate colony loss and to optimise preventive treatments. Studies have shown that the viability of *Nosema* spores is influenced by temperature and the differential distribution of the two *Nosema* species in Europe indicates an influence of climate. The data used comes from a study of about 250 colonies from 25 apiaries in the North-east Germany monitored twice annually since 2005, thrice since 2009. The incidence and infection levels of both *Nosema* spp. were analysed and compared against daily weather variables. Aggregates of weather variables for different time window sizes (10-180 days) were extracted for the months prior to the collection of the bee samples. The relationship between the aggregated weather variables and the pathogens were analysed using data mining and statistical tests. The results of analysis will be presented.

DB-6 STU **A cell line resource derived from honey bee (*Apis mellifera*) embryonic tissues.**

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It is not uncommon for honey bee colonies to succumb to the culmination of the negative effects from mite pests, pathogens, pesticides, and poor nutrition. It has become an important research effort to mitigate these challenges to honey bee health and colony productivity. A major hindrance to the study of honey bee pathogens or the effects of pesticides and nutritional deficiencies is the lack of controlled *in vitro* culture systems comprised of honey bee cells. We have developed a method incorporating established insect cell culture techniques that supports sustained growth of honey bee cells derived from embryonic tissues. Serial transfer of material from several primary cultures was maintained and has led to the isolation of young cell lines. A cell line has been established that is composed mainly of fibroblast-type cells that form an adherent monolayer. Most cells in the line are diploid (2n = 32) and have the *Apis mellifera* karyotype as revealed by Giemsa stain. The partial sequence for the mitochondrial-encoded cytochrome c oxidase subunit I (Cox 1) gene in the cell line is identical to those from host tissues and a consensus sequence for *A. mellifera*. Importantly, the cell line is continuously subcultured and is cryopreserved in liquid nitrogen. We also present preliminary findings from infection experiments with the emerging fungal pathogen, *Nosema ceranae*. The cell culture system we have developed has potential application in studies aimed at honey bee genetics, pathogenesis, transgenesis, and toxicology.

DB-7 STU **Analysis of the relationship between the honey bee, *Apis mellifera* and the ectoparasite *Varroa destructor*.**

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The honey bee, *Apis mellifera*, is an extremely important economic insect, responsible for around 80% of the global agricultural pollination services. The closely interacting and densely populated colonies of up to 50,000 individuals provide an optimum environment for the proliferation of various pathogens and parasites, and the implications of this are extremely important for the survival of the honeybee. One of the most serious threats to the survival of honey bee colonies is the hemophagous mite, *Varroa destructor*. It is an ectoparasite that attaches itself to the cuticle of both the larval and adult stage of the bee, feeding on the haemolymph. The effects of parasitization by *V.destructor* are detrimental, with a negative effect on the physical health of the bee due to loss of haemolymph, but also on the immune system of the larvae, due to immune suppression and the transmission of viral particles around and between colonies. Also, in recent years, *V.destructor* has developed resistance to one of the two products licensed for use in Europe for the treatment of colonies. Through proteomic analysis, the relationship between *A.mellifera* and *V.destructor* was examined, with emphasis on the effect of parasitization on the immune system of *A.mellifera* larvae. The possibility of transmission of viral particles from the feeding of haemolymph by *V.destuctor* was also examined, with RT-PCR analysis of six bee viruses to indicate the presence or absence of viruses in the mites and the honeybee.

F-1 **Granulate ambrosia beetle survival and brood production following exposure to entomopathogenic and mycoparasitic fungi**

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The granulate ambrosia beetle *Xylosandrus crassiusculus* is among the most important exotic pests of orchards and nurseries in the US. The beetle has a wide host range and is difficult to control using conventional insecticides because of it cryptic habits. The use of microbial agents, specifically entomopathogenic and mycoparasitic fungi, is largely unexplored, but may prove effective by targeting females, or foundresses, and their brood inside tree galleries. In this study we tested the susceptibility of *X. crassiusculus* to commercial strains of entomopathogenic fungi *Beauveria bassiana*, strains GHA and Naturalis, and *Metarhizium brunneum*, strain F52. We also evaluated brood production among foundresses exposed to these biocontrol agents or the mycoparasitic fungus *Trichoderma harzianum.* Bioassay studies showed that strains GHA, Naturalis, and F52 were virulent against females, with a mean LD_{50} of 280 ± 177 conidia/mm^2, 85 ± 30 conidia/mm^2, and 232 ± 110 conidia/mm^2, respectively, 5 days after spraying. Foundresses exposed to beech stems treated with entomopathogenic fungi at the highest dose had lower survival rates and produced fewer galleries. All doses tested, however, resulted in females with smaller brood size compared to control. Those exposed to *T. harzianum* produced galleries with sparse, patchy, or no symbiont growth and fewer brood. Complementary studies also showed negative interactions between these biocontrol fungi and the beetle symbiont. These results demonstrate that beetle populations may be targeted directly by killing foundresses or indirectly by suppressing growth or establishment of the fungal symbiont beetles culture as a food source.

F-2 **Tracing the origin(s) of exotic woodwasp *Sirex noctilio* via genetic studies of its fungal symbiont *Amylostereum areolatum***

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The wood wasp *Sirex noctilio* is a serious pest of pine plantations in the Southern Hemisphere, causing up to 80% tree mortality during heavy outbreaks. The wasp is native to Europe, but has spread worldwide during the course of the last century. It was first discovered in North America in New York in 2004, and since then has been collected from Michigan, New Jersey, Ohio, Pennsylvania, Vermont, Ontario and Quebec. *Sirex noctilio* is associated with a symbiotic white rot fungus, *Amylostereum areolatum*, which females inject into trees when they oviposit and which is required for larval development. Current biological control strategies against this pest utilize the nematode *Deladenus siricidicola*, which parasitizes *S. noctilio* as well as feeds on *A. areolatum* for parts of its life cycle. The effective use of this nematode requires testing the suitability of different strains of *D. siricidicola* against different genotypes of the *Sirex-Amylostereum* complex, since the nematode-wasp association may be specific to certain geographic populations. In this study we examined the genetic diversity of *A. areolatum* isolated from *S. noctilio* from Europe in comparison with samples from New York. Multilocus genotyping (ITS, mtssu, RPB2, tef1, and laccase genes) revealed multiple fungus genotypes in New York: one genotype represented one of two more common genotypes found in Europe and the rest were unique, which suggested unrepresented source populations. These results concur with reports of multiple introductions of *S. noctilio* to North America and suggest that more than one strain of *Deladenus* spp. may be required for control of this pest in the US.

F-3 Conidia of the fungus *Neozygites floridana* are forcibly discharged over a relatively short period

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One of the most important natural enemies of the two-spotted spider mite, *Tetranychus urticae*, is the fungus *Neozygites floridana*. To facilitate the use of this pathogen through inoculative releases it is still necessary to understand important ecological and biological aspects of the fungus. The minimum period of high relative humidity (RH) for sporulation is an important information to develop large scale production of the fungus *in vivo* and to determine timing for field releases. These parameters were determined in the laboratory by placing *N. floridana* killed cadavers from a Brazilian isolate (ESALQ 1420) to sporulate on Jack-Bean leaves (*Canavalia ensiformis*) in the dark in climatic chambers at 90, 95 and 100% RH and 13ºC and 25ºC. Most of the sporulation occurred like a burst between 8h and 12h at RH≥90% at all temperatures. The fungus did not sporulate within the first 4h and the sporulation was insignificant after 12h. For this Brazilian *N. floridana* isolate, sporulation was 10 times greater at 25ºC compared to 13ºC. Lower temperature optimums have, however, been seen for *N. floridana* isolates from the Northern hemisphere. The RH had a great influence on sporulation. At 25ºC and 90% RH, an average of 277 primary conidia and 306 capilliconidia per mummified mite were produced and at 100% RH and 25ºC, 131 primary conidia and 1101 capilliconidia were observed. This demonstrates that the sporulation and germination for this Brazilian *N. floridana* isolate is greatest at ≥95% RH and at 25ºC.
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F-4 Characterizing the diversity of entomopathogenic fungi in Brazil

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This project aimed to increase knowledge about the diversity and abundance of entomopathogenic fungi in the Brazilian biomes. Soil samples were collected in areas of native vegetation, annual and perennial crops in five biomes, Amazon, Caatinga, Atlantic Forest, Cerrado (Savanna) and Pampa. Isolation of entomopathogenic fungi was performed by selective medium and "Insect bait" using *Galleria mellonella* and *Tenebrio molitor*. In the first sampling conducted in 2012, it was noted in the Cerrado and in corn fields in the Amazon, a greater amount of *Metarhizium* sp. In the Caatinga, *Beauveria* sp. was collected in greater quantities than other fungi. The genus *Isaria* was found in the Amazon, Caatinga and Cerrado and *Lecanicillium* sp. only in native vegetation of the Cerrado but with a low number of CFU for both fungi. A total of 500 fungal isolates were preserved from only one sampling period and the ones identified by sequencing belong to the species *M. anisopliae*, *M. robertsii*, *M. majus*, *B. bassiana* and *Isaria fumosorosea*. All *Metarhizium* based product in Brazil consist of *M. anisopliae* s.s. In surveys carried out in three geographic area of the state of Sergipe (sertão, agreste and east), there was a higher occurrence of entomopathogenic fungi in soils with a pH between 5.1 to 6.0 and organic matter exceeding 0.7%. In the oil base of the Urucú River in the Amazon forest, 69 isolates of *Trichoderma* were obtained from the species *T. spirale*, *T. virens*, *T. harzianum* and *Trichoderma asperellum*, the latter being the predominant species. Sampling will be carried out during the raining and the dry season for two years for better characterization of the diversity of these widely distributed fungi in Brazil.

F-5 Abiotic and biotic factors affecting resting spore formation of the mite pathogen *Neozygites floridana*

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Neozygites floridana is an obligate mite pathogenic fungus in the Entomophthoromycota. It has been suggested that resting spores of this fungus are produced as a strategy to survive adverse conditions. In the present study, possible mechanisms involved in the regulation of resting spore formation were investigated in the hosts *Tetranychus urticae* and *Tetranychus evansi*. Abiotic and biotic factors mimicking adverse conditions in temperate and tropical regions were tested with isolates from Norway and Brazil to induce resting spore production. A total of 42 combinations of conditions were tested, but only one condition stimulated the formation of a high number of resting spores, and this occurred in only one isolate. The Brazilian isolate ESALQ1420 produced a large number of resting spores (51.54%) in *T. urticae* at a temperature of 11°C, photoperiod of 10L:14D, and light intensity of 42–46 (µmol m-2s-1) on non-senescent plants (non-diapausing females). Small percentages of *T. urticae* with resting

spores (0–4.65%) were found for the Norwegian isolate NCRI271/04 under the conditions tested. The percentages of resting spores found for the Norwegian isolate in our laboratory studies are similar to the prevalence reported in earlier field studies. These findings support the hypothesis that the main overwintering strategy for *N. floridana* in temperate regions is to form hyphal bodies inside live hibernating *T. urticae* females rather than to form resting spores. It also seems unlikely that resting spore formation is the major strategy for surviving adverse conditions in tropical climates, given that the conditions that best induced resting spore formation by the Brazilian isolate are not common in the tropics.

F-6 Seasonal variation in phenoloxidase and pro-phenoloxidase activity in *Phyllophaga polyphylla* inoculated with blastospores of an entomopathogenic fungus

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Insects developing in the field with sufficient resources and optimal environmental conditions are expected to have more resources to invest in immunity than those raised in adverse conditions. The relationship between environmental conditions and immune response was examined in field-collected larvae of the white grub *Phyllophaga polyphylla*. Third-instar *P. polyphylla* larvae were collected from maize fields in October 2011 and 2012. Different groups of larvae were inoculated with one of two treatments, either viable or non-viable blastospores of the entomopathogenic fungus *Metarhizium pingshaense*. For each treatment 1 X 10^3 blastospores were injected into each larva. Control larvae were injected with PBS. Hemolymph samples were obtained from each larva 12 and 24 h after inoculation and the prophenoloxidase (proPO) and phenoloxidase (PO) activity measured, both of which are important components of the insect immune response and frequently used to measure immune defence ability. There were differences in PO activity between 12 and 24 h, but not in proPO activity. There were also differences in PO and proPO activity between larvae collected in different years. Highest PO and proPO values were found in the year with more rainfall, which provided better conditions for the maize to grow, and therefore provided more resources to *P. polyphylla* larvae to feed. Our data suggest that seasonal variation in conditions for larvae to grow will influence the effectiveness of the immune response. These variations could affect the efficacy of entomopathogenic fungi used for the control of *P. polyphylla*.

F-7 Conidial vigor (robustness) affects virulence of *Beauveria bassiana*

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For many entomopathogenic fungi, viability (% living conidia) can be determined through germination assessments performed after an extended period of incubation (≥48 h) at 25ºC on an agar-based substrate containing a fungistatic compound. On the other hand, for most *Beauveria bassiana* isolates, vigor (% conidia capable of rapid germination) can be estimated by incubation on standard PDA for 16-18 h. We tested the hypothesis that conidial vigor is a better predictor of virulence than viability. We performed bioassays with 3rd-instar *Spodoptera frugiperda* larvae (Lepidoptera: Noctuidae) and *B. bassiana* batches (isolate CG1027) with contrasting viabilities (65 vs. 96%) and vigor (4 vs. 93%, respectively). For suspensions with similar concentrations of viable conidia, mortality of insects was consistently higher and faster in treatments with a greater proportion of fast-germinating conidia. The same trend was evident when bioassays were performed with two other conidial batches displaying slightly different viabilities (79 vs. 89%), but contrasting vigor (45 vs. 83%). Regarding suspensions with similar concentrations of vigorous conidia, we observed that (1) for batches with slightly different viabilities, virulence was similar; (2) for batches with contrasting viabilities, estimated LC50 for the lower-quality batch (slow-germinating: fast-germinating conidia, ratio > 15) was considerably smaller. This result seems to be explained by the fact that slow-germinating conidia are pathogenic to *S. frugiperda*, although considerably less virulent than fast-germinating propagules. We argue that 'conidial vigor' is a critical parameter to be considered in protocols designed to assess the quality of biopesticides based on *B. bassiana* and possibly other fungi.

F-9 Are secondary metabolites dispensable for virulence?

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The production of toxins by conidial fungal pathogens and their association with virulence has been assumed to occur *in vivo* and is widely accepted as dogma, but this association has yet to be definitively proven by either genetic or chemical means. Several studies from our labs have used targeted gene disruption approaches to directly address the role of secondary metabolites (SM) in insect pathogenesis, using *Metarhizium robertsii* ARSEF 2575. Knock out mutants for three distinct secondary metabolite pathways- destruxins, the fusarin analogs NG391/NG393, and the conidia-localized serinocyclins- showed wild-type level virulence against several insect host species. No phenotypic differences in morphology, growth, development or response to oxidative stress were exhibited by the knockout strains. These findings are in stark contrast to the outcome predicted by a large body of *in vitro* toxicological studies suggesting SMs, such as the destruxins, are key virulence factors for *Metarhizium* invertebrate pathogens. We suggest that the relationship of SMs to virulence may be coincidental, and the compounds may play roles in other unknown functions, or cause subtle effects in the host, not measurable with traditional pathogenicity assays. However, given the range of potentially detrimental activities reported for these SMs, including mutagenicity, ionophoresis, and cytotoxicity, their potential risk as environmental contaminants or pollutants remains a concern and the availability of SM- strains that retain virulence against insects provides a pool from which to draw for the engineering of safe and effective biocontrol strains.

F-10 Mycoses of European fruit lecanium scale

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European fruit lecanium scale (LS), *Parthenolecanium corni* Bouche, is an important factor in the decline of leaf-bearing trees in the USA and Canada. LS is invasive pest and study of local biotic factors limiting of insect populations is very important as a basis for the integrated pest management strategy. The research of natural insect mortality cause of entomopathogenic fungi and study of pathogens was concentrated on the three basic directions including isolation of

fungi from LS; determine the artificial media for cultivation of specialized entomopathogenic fungi and estimation of efficacy of fungi against pest. Numerous cultures of entomopathogenic fungi including *Hirsutella* sp., *Beauveria bassiana, Lecanicillium muscarium, Paecilomyces farinosus, Trichoderma* sp., *Fusarium* sp., *Gliocladium* sp. and some others were isolated and deposited in ARS USDA and UVM fungal collections. The special attention was paid to fungus *Hirsutella* sp. as specialized pathogen of scales. The basic cultural and others biological properties of fungus were estimated. The best isolates produced from 4.5 to 12.3 x 10^6 conidia per one square centimeter of solid medium and biomass accumulation was 9.3±0.3 and 10.4±0.4 per 100 ml liquid medium after 120 and 240 hours cultivation respectively. Selected branches of maple trees were treated using suspension of air conidia with concentration 5 x 10^7 per ml. External signs of mycosis were observed among 35% scales after observation in the period two month.

F-11 ***Hirsutella myrmicarum*, a new species infecting invasive *Myrmica rubra* in Maine**

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Myrmica rubra L. (Formicidae) is an invasive ant species in Maine and has been the subject of recent studies into the natural mortality and management of invasive species. Over several years we have observed a fungus growing from moribund *M. rubra in vitro* that has not previously been reported, and we have isolated multiple cultures over 2 y. We analyzed the morphology of this fungus and its placement within molecular phylogenies of four genomic DNA regions. We identified it as a member of the genus *Hirsutella* Pat. (Ophiocordycipitaceae; Hypocreales). Molecular and morphological characters support the description of this fungus as a new species, *H. myrmicarum*, which is the first in this genus to be isolated from North American *M. rubra*. Though sporulation of the fungus on the host occurs somewhat rapidly after the ant is deceased, sporulation can take months to occur on agar media. By monitoring growth on media with different sugar content or nutrient origins, we expect to find a manner to grow the fungus in mass quantities for non-target vector analyses.

F-12 First report of mycoparasitism of entomophthoralean resting spores

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Mycoparasitism–when one fungus parasitizes another–has been reported to affect *Beauveria bassiana* and mycorrhizal fungi in the field. However, mycoparasitism of any fungi in the Order Entomophthorales has never before been reported. The majority of entomophthoralean species persist as resting spores (either zygospores or azygospores) in the environment. Dormant entomophthoralean resting spores (whether formed as zygospores or azygospores) are thought to be especially well adapted for survival over long periods due to their thick double walls, and they can accumulate in the soil as large reservoirs of inoculum that can facilitate the onset and development of epizootics. We report parasitism of resting spores of the gypsy moth pathogen *Entomophaga maimaiga* caged in soil from Ohio by the chytrid fungus *Gaertneriomyces semiglobifer*. *G. semiglobifer* had previously been isolated from soil samples from North America, Europe and Australia or horse manure from Virginia. After isolation and identification of *G. semiglobifer*, resting spores of *E. maimaiga* exposed to zoospores of *G. semiglobifer* showed high levels of mycoparasitism. *G. semiglobifer* was subsequently reisolated from mycoparasitized resting spores. We discuss the importance of this finding to the epizootiology of insect diseases caused by entomophthoralean fungi.

F-13 Three *Metarhizium anisopliae* isolates to control of *Spodoptera exigua.*

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Beet armyworm, *Spodoptera exigua* are difficult to control using chemical insecticides because of the development of insecticide resistance. For eco-friendly beet armyworm managements, various control agents are required. Entomopathogenic fungus is one of promise control agents as an alternative to chemical pesticides. One hundred fifty isolates, which were collected from soil samples of nine counties in Korea by insect-bait method using *Galleria mellonella* and *Tenebrio molitor*, were used for bioassays to select high virulence isolate to larva of beet armyworm. As a result of first screening, three fungal isolates showed high pathogenicity against *S. exigua*. These isolates were identified as *Metarhizium anisopliae* by microscopic examination and genetic sequencing of 18srRNA. Three isolates killed the pest by direct fungal infection and/or starvation which may be caused antifeedant effect. We are concluding that these isolates are candidate to develop mycopesticide to control beet armyworm in crop productions.

F-14 Population Genetics of *Beauveria bassiana* Isolates from U.S. Northern Plains Grasshoppers.

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In August 2010 and August 2011 we made 307 isolations of *Beauveria bassiana* from diverse Melanoplinae and Gomphocerinae grasshoppers at 5 locations in western North Dakota and Eastern Montana. Prevalence of B. bassiana infections among live grasshoppers in the single collection ranged 5-18% among the 2010 sites and 7-12% in 2011. Single spore isolations were performed because in previous work we observed natural multiple infections in individual grasshoppers. These isolates, typically 28 - 118 in number at each location, were first subjected to analysis using the *Bloc* gene sequence for assignment into clades. They were then further assessed using AFLP. Based on the Bloc gene, the isolates represented 15 distinct groupings, falling into 7 clades. These clades were copresent at all the sites, typically 5-10 at any one site, but in varying proportions. AFLP haplotyping further distinguished a large degree of genetic variability. There was no distinct association of clade or AFLP haplotype with host grasshopper species.

F-15 Molecular and biochemical characterization and virulence of *Metarhizium* spp. of canadian origin against emerald ash borer.

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The emerald ash borer, *Agrilus planipennis* (EAB) is an invasive wood boring beetle that is decimating North America's ash trees (*Fraxinus* spp.). To find effective and safe indigenous biocontrol agents to manage EAB, we conducted a survey in 2008-2009 to recover entomopathogenic fungi infecting EAB from outbreak sites in

Ontario, Canada. Four *Metarhizium* isolates were retrieved from dead and mycosed EAB cadavers and additional 4 isolates were isolated from soil samples in the vicinity of the sampled sites. Molecular characterization with generated ITS, 5' end of EF1-α sequences confirmed the identity of the isolates as *M . robertsii*. A total of 14 isolates including EAB recovered as well as other indigenous *Metarhizium* sp. viz., *M. robertisii*, *M. brunneum* and *M. flavoviride* were tested for their virulence against adult EAB. A dose of 2×10^6 conidia/ml for each isolate was used to estimate mean survival time (MST). All the *Metarhizium* isolates were pathogenic to adult EAB but with varied virulence. One of the soil isolates *M. robertsii* MABli killed EAB adults faster than all other isolates with a MST of 4.80 d, however did not significantly differ from the EAB isolate W3aA (5.11 d) (Log-rank test, $p>0.05$). Based on their virulence, further biochemical characterization, conidia production *in vivo* and *in vitro* were conducted with 8 selected isolates. The virulence of MABli is also observed to be linked with high production of ammonia by-products based on visual observations. Further quantitative analysis including the secretion of Pr1- and- Pr2-like proteases will be presented.

F-16 Linking phylogeny and taxonomy: a molecular revision of *Metarhizium*

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The genus *Metarhizium* traditionally refers to green-spored asexual insect pathogenic fungi. Through the use of culturing and molecular methods, *Metarhizium* has been linked to *Metacordyceps* sexual states. Historically, fungal nomenclature allowed separate names for the different life-stages of pleiomorphic fungi. However, with the move to one name for one fungus, regardless of life-stage, there is a need to determine which name is the most appropriate. The situation is complicated by the fact *Metacordyceps* sexual states are interspersed among additional asexual genera, including *Pochonia*, *Nomuraea* and *Paecilomyces*. *Metarhizium* has priority as the earliest published available name, but delimiting the boundaries of this genus remains problematic. Previous phylogenetic work identified a core *Metacordyceps* clade, comprised of strongly pigmented species including the asexual genera *Metarhizium* and *Nomuraea*, subtended by a poorly resolved grade of species lacking pigmentation and including asexual forms identified as *Pochonia* and *Paecilomyces*. In an effort to clarify relationships among these taxa we have obtained representative material for each genus and established a molecular dataset of five protein-coding genes. The resulting phylogeny supports *Metarhizium* containing the majority of species recognized in *Metacordyceps* as well as the green spored species in *Nomuraea*. *Pochonia* was found to be polyphyletic, and we recognize the genus to contain *P. chlamydosporia* and *P. catenulata* stat. nov., with the remaining species moved to *Metapochonia* gen. nov. It is our hope that this unified concept of sexual and asexual states in *Metarhizium* will foster advances in understanding the unique ecologies of the associated species.

F-17 Toxicity of culture filtrates of entomogenous fungi to cotton aphid and green peach aphid

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Some disadvantages of mycopesticides such as no knoc-down effect and inactivation by environmental factors are causing that mycopesticide market doesn't increase. Especially, conidia of entomopathogenic fungi is influenced by environmental conditions such as temperature and relative humidity and caused slow and fluctuation of mortality. To solve the unstable and slow control effect we try to use fungal culture filtrates which may have secondary metabolites. Bioassays were conducted with culture extracts of 47 entomogenous fungi to screen isolates having high toxicity to aphids. Fungal isolates were cultured for 3, 5, 7, and 10 days in PDB and the culture broths were filtrated through filter paper and syringe filter. The extracts were sprayed onto leaf discs having aphids. Most of culture filtrates from *Cordycep* spp. had low or no insecticidal activity. Other culture filtrates from different isolates and different culture periods exhibited different control effects. Culture filtrate of an isolate of *Beauveria bassiana*, especially 5 days culture filtrate, showed high toxicity against cotton aphid and green peach aphid as LT_{50} at 2.7 and 2.0 days, respectively. These results indicated that the 5 days culture filtrate of an isolate of *B. bassiana* has potential to be developed as biochemical or microbial pesticide for aphid control.

F-18 *MrInv*, encodes an extracellular invertase in *Metarhizium robertsii*, facilitates rhizosphere competency during *Metarhizium*-plant associations

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The soil-inhabiting insect-pathogenic fungus *Metarhizium robertsii* establishes mutualistic symbiosis with many plants. As well as killing pest insects, *Metarhizium* also boosts plant growth by providing nitrogenous nutrients and increases resistance to plant pathogens. In rhizosphere, plant roots secret abundant nutrient to support microbial population. However, little is known about how *Metarhizium* utilize such plant-derived nutrient and the mechanistic basis of *Metarhizium*-plant associations. We report here that an extracellular invertase, the product of *MrInv* gene, was identified and characterized important in the molecular level that controls the rhizospheric interaction and fungal growth during *Metarhizium*-plant associations. In silico analysis revealed MrINV contained a secretion signal peptide and the enzymatic activity assay confirmed the extracellular localization of the protein. Deletion of *MrInv* (⊿*MrInv*) specifically reduced *M. robertsii* growth on sucrose and rhizosphere competence. However, it increased plant root colonization and resulted in higher virulence to the insect *Galleria mellonella*. An increased production of depolymerases active against plant cell walls and insect cuticles by ⊿*MrInv* may have increased invasiveness to plant and insect hosts.

F-19 Evaluation of soil microfungi as biological control agents against eggs of animal parasitic nematodes

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Thick-shelled eggs of ascarid nematodes have been reported to remain infective in the environment for several years, thus posing a prolonged risk of infection to animal livestock and/or humans. An *in vitro* study was therefore conducted to evaluate the negative impact of two species of soil microfungi, *Pochonia chlamydosporia* and *Purpureocillium lilacinum* (syn. *Paecilomyces lilacinus*), on the viability of *Ascaridia galli*, *Toxocara canis* and *Ascaris suum* eggs. Approximately 150 fresh eggs of individual ascarid species were embryonated on a 2% water agar in Petri dishes with or without a fungus (*P. chlamydospria* or *P. lilacinum*). On days 7, 14, 21, 28, 35 and 42 post experimental set up (p.s.), the viability of the eggs from each experimental group was evaluated (destructive sampling). By day 14 p.s., *P. chlamydosporia* had reduced the viability of *A. galli* and *T. canis* eggs by 70-86% and 52-67%, respectively, compared to the controls. In contrast, *P. lilacinum* had reduced the viability of *A.*

galli and *T. canis* eggs by only 17-30% and 6-28%, respectively. Neither fungal species was found to be effective against *A. suum* eggs (<4% reduction in both cases). These results indicate interspecies differences in the susceptibility of ascarid eggs to soil microfungi. *Ascaridia galli* and *T. canis* eggs seemed to have been degenerated mainly due to hydrolysis of shells by fungal enzymes. The present study demonstrates that *P. chlamydosporia* may potentially be utilized as a biological control agent against *A. galli* and *T. canis* egg contaminations in the soil environment.

F-20 Entomopathogenic fungi of the genus *Cordyceps* and their insect host range

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Cordyceps, a genus of entomopathogenic fungi, is known to produce useful bioactive compounds; therefore, it has long been studied in various fields. In 1990, there was remarkable development in the study of *Cordyceps* in Korea; *Cordyceps militaris* was successfully cultivated artificially by using the silkworm as an insect host. Recently, *Cordyceps* has been studied for the development of a biological control agent as well as pharmacological use. It is possible by a reason that *Cordyceps* has host specificity, the various species that infect the host optionally. Accordingly, securing and preserving a variety of wildlife resources is very valuable for future use in order functional studies.This experiment was performed to introduce several resources by analyzing the characteristics of entomopathogenic fungi and to use these resources as basic data for taxonomic studies in the future. we investigated the characteristics of 21 specimens of entomopathogenic fungi originating from Tingo Maria, Peru. Of these, 11 specimens accounted for 52% of the parasites on insect hosts of the order Diptera; 2 parasitized hosts of the order Araneae; 2, order Othoptera; 1, order Lepidoptera. Five of the specimens could not be identified because the host insects or fruiting bodies were decayed. The species were identified as *Cordyceps discoideocapitata*, *Beauveria bassiana*, and *Cordyceps* sp. After the strains of unidentified fungi are identified, all the specimens will be subject to pathogenicity tests and a mass production experiment using silkworm larvae as a host.

F-21 Detoxification system induction, antioxidant response and oxidative stress levels in the entomopathogenic fungus *Beauveria bassiana* exposed to the pyrethroid deltamethrin

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The chemical control of the Chagas disease vector *Triatoma infestans* is endangered by the emergence of pyrethroid resistance. Among alternative control tools, laboratory and field assays showed the efficacy of the entomopathogenic fungus *Beauveria bassiana* strain GHA to kill *T. infestans*. Several fungal isolates were shown to tolerate well deltamethrin doses similar to those used for *T. infestans* control, providing evidence that the combination of deltamethrin with entomopathogenic fungi might be used in future control strategies.
In this work, the effect of deltamethrin on fungal growth, gene expression and enzyme activity in relation to detoxification, antioxidant response and oxidative stress levels was studied to evaluate fungal tolerance to deltamethrin. The mean inhibitory concentration (IC_{50}) was 50 µg deltamethrin/cm^2. Cytochrome P450 genes were differentially expressed; *cyp52X1* and *cyp617N1* transcripts were > 2-fold induced, followed by *cyp655C1* (1.8-fold). Minor effects were observed on genes encoding for other P450s, epoxide hydrolase and glutathione S-transferase (*gst*). Superoxide dismutase (*sod*) genes showed induction levels ≤ 2-fold, catalase (*cat*) and glutathione peroxidase (*gpx*) genes were also induced ~ 2-3-fold and < 2-fold, respectively. The activities of enzymes participating in the antioxidant defense system and phase II detoxification were also evaluated; SOD, CAT and GST activity showed significant differences with deltamethrin concentration. Lipid peroxidation levels and free proline content were also altered. We conclude that *B. bassiana* strain GHA can be used combined with deltamethrin without significant metabolic detrimental effects. This combination will help optimizing the benefits and increasing the efficacy of vector control tools.

F-22 Visible light during mycelial growth of entomopathogenic fungi increase conidial tolerances to heat and oxidative stress

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Visible light exposure during growth influences primary and secondary metabolism, growth and sporulation, sexual and asexual development, and pigment formation in many fungal species. However, little is known about the phenotypic effects of light during mycelial growth on the tolerance of the developing fungal conidia to different stress conditions. Conidia of the entomopathogenic fungi *Beauveria bassiana* (ARSEF 252), *Metarhizium brunneum* (ARSEF 1187), *M. robertsii* (ARSEF 2575), *Tolypocladium cylindrosporum* (ARSEF 3392), *Isaria fumosorosea* (ARSEF 3889), *T. inflatum* (ARSEF 4877), *M. anisopliae* s.l. (ARSEF 5749), *Lecanicillium aphanocladii* (ARSEF 6433), *Simplicillium lanosoniveum* (ARSEF 6651), and *Aschersonia aleyrodis* (ARSEF 10276) were produced on PDA medium under continuous light, on PDA medium in the dark, and on minimal medium in the dark (MM). The conidial tolerance of these species was evaluated in relation to heat and to menadione, a potent inducer of reactive oxygen species. The conidial production under the three treatments was also evaluated. Visible light during mycelial growth increased heat tolerance only in conidia of ARSEF 5749. The tolerances to menadione were higher for the isolates ARSEF 1187, ARSEF 2575, and ARSEF 5749 when conidia were produced under light. The nutritive stress caused by MM induced increased heat tolerance to conidia of ARSEF 1187, ARSEF 2575, ARSEF 4877, and ARSEF 5749. Conidia produced under nutritive stress had higher tolerance to menadione for the isolates ARSEF 252, ARSEF 1187, ARSEF 2575, ARSEF 3392, and ARSEF 5749. Increased conidial production was induced by visible light only for the *Tolypocladium* species ARSEF 3392 and ARSEF 4877.
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F-23 A solid formulation of *Beauveria bassiana* (isolate 203) against Red Palm Weevil (*Rhynchophorus ferrugineus)*

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The Red Palm Weevil (RPW, *Rhynchophorus ferrugineus*) is causing a devastating epidemics in the Mediterranean area, Middle East countries and recently it has been introduced in North America (California). Unfortunately, until today no effective means to control RPW are available. An isolate of *Beauveria bassiana* (Bb203) has been found infecting naturally RPW adults in SE Spain. A solid formulation was developed for RPW biocontrol. *Beauveria bassiana* isolates from several origins have been tested under laboratory, semi-field and field conditions. *Beauveria bassiana* isolate 203 obtained from RPW in field was the most effective one controlling the palm pest. For field assays, a 5-levels visual scale has been created to evaluate RPW symptoms in Canary palms. Field assays were conducted in 2009 and 2011 at the same plot (3 treatments/year). The results showed that Bb203 formulation has a high capacity to control RPW in field palms (90%-70% RPW mortality in 2009 and 2011 respectively). RPW adults with *B. bassiana* signs were found in both treated and untreated palms. Insects with Bb203 signs were found at treated plot in 2010 (12 months after last treatment). A higher percentage of palms with low levels of infestation (1-2) was found in Bb203 treated palms compared with respect to those untreated (47%-23% respectively). This study has shown that a mycoinsecticide based on *B. bassiana* 203 is an effective method to control RPW under field conditions. An Integrated Pest Management program will be implemented with this biological control agent against this pest.

F-24 Defensive role of hemolymph in the silkworm, *Bombyx mori* to *Beauveria bassiana*

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The white muscardine of silkworm caused by the silkworm, *Bombyx mori* infected with *Beauveria bassiana*, a pathogenic fungus, was widely occurrence in all sericultural area and every silkworm rearing season, and is one of the major infectious diseases that threat to sericulture production. When the survival larva of silkworm were injected by 1.8×10^5 cells/mL *Beauveria bassiana* conidia at 3 days in fifth instar, one or multiple conidia adhered on the surface of haemocytes and the pseudopodia-like projections extend from haemocytes could be observed after 15 min of the injection. The haemocytes phagocytized conidia and circle vacuoles around the phagocytized conidia could be observed after 6 h of the injection. After 12 h of injection, black agglomeration which gathered by haemocytes surrounded conidia can be observed on the surface of fat body, muscles, digestive tract and tracheae. The conidium phagocytized or surrounded by haemocytes begin to germinate and the germination mycelium extending outside from agglomeration or haemocytes after 24 h of injection. The results showed that the haemocytes of the silkworm were capable of phagocytizing and encapsulating *Beauveria bassiana* conidia to form the melanized nodules, but its fail to suppres the conidia germination in the nodules, only take a temporary defense response. When the *Beauveria bassiana* conidia and the silkworm haemocyte were mixed in whole silkworm plasma, the numbers of conidia adhering on haemocytes were significantly more than the mixing with the silkworm plasma pre-absorbed by *Beauveria bassiana* conidia and the saline without the silkworm plasma, were respectively 2.16 times and 2.51 times of the latter two. When the *Beauveria bassiana* conidia was pretreated with the silkworm plasma and subsequently mixed with haemocytes in the saline, the numbers of conidia adhering on haemocyte was 1.56 times of the no pre-treatment. The MW 64, 40.3, 36.9, 35.2, 33, 26.7 and 25.8 ku protein components were disappeared, the MW 60.0 and 28.5 ku protein components were weakened in silkworm plasma treated repeatedly with high concentration of *Beauveria bassiana* conidia. Above disappeared and weakened protein components consists with plasma protein components adsorbed on surface of the conidium. The results showed that the silkworm plasma had accelerative activity on recognition and adhesion of the *Beauveria bassiana* conidia by the silkworm haemocytes. One or more protein components (MW 64, 60, 40.3, 36.9, 35.2, 33, 28.5, 26.7, 25.8 ku) in the silkworm plasma were bound on the conidia before its accelerative activity took place.

F-25 Karyotype analysis and protoplasts characteristic of *Beauveria bassiana*, a pathogenic fungus of *Bombyx mori*

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Beauveria bassiana, a pathogenic fungus of *Bombyx mori*, is isolated as an infectious pathogen of sericulture, however is utilized as a fungus resource of producing *Bombyx batryticatus*, a traditional Chinese medicine. The gentle shaking treatments at 30℃ for 1.5 hours with Driselase (6 mg/mL) as enzyme solution and the 0.7mol/L NaCl (pH 5.8) as osmotic stabilizer were concluded to be the most suitable conditions for isolating protoplasts from young mycelia of *Beauveria bassiana* of silkworm. The protoplasts of *Beauveria bassiana* of silkworm were cycloid, and had strong refraction. Among the protoplasts, 26.5% were without a nucleus, 53.5% contained a single nucleus, 13.5% contained two nucleus, and 6.5% ciontained three or more nucleus. After 12 hours of incubation in L-broth medium at 28℃, the protoplasts begin to regenerate and reverse into mycelium. Three morphologically distinct regeneration and reversion types of the protoplasts were observed as follows: ① stretched out myceliumgermination tubular directly from the protoplast, ② stretched out beaded chain like yeast bud from protoplast, then extend mycelium from the top of beaded chain, ③ stretched out beaded chain like yeast bud from protoplast, but the top of beaded chain does not extend mycelium with itself dissolve away. The regenerating protoplasts and the no-regenerating protoplasts could not be distinguished under a phase contrast microscope. Osmotic stabilizer of medium has significant influence to regeneration and reversion of protoplasts. Through the comprehensive survey of the colony formation of protoplasts and the growth of mycelium, it is suggested that 0.7 mol/L glucose is more suitable than sodium chloride, potassium, mannitol and sorbitol as osmotic stabilizer for regeneration and reversion of protoplasts. Chromosomal DNA samples were prepared with protoplast of *Beauveria bassiana* of silkworm, and it's karyotype were investigated by counter-clamped homogeneous electric field (CHEF) gel electrophoresis technique of pulsed-field gel electrophoresis. *Beauveria bassiana* of silkworm had at least six chromosomes, whose sizes were estimated to be 6.6, 5.6, 4.7, 3.9, 3.2 and 2.5 megabase pairs (Mbp) respectively, and the karyotype's sizes were estimated to be. 26.5 Mbp. Significant differences in the size of each chromosome existed among the isolates of *Beauveria bassiana* of silkworm.

F-26 Fungal entomopathogens as endophytes in biological control for beans and cassava

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The common bean (*Phaseolus vulgaris*) and cassava (*Manihot esculenta*) are essential food crops in tropical countries throughout the world. Yields can be severely reduced by insects, making pest management an important component in achieving an adequate food supply. Endophytic fungal entomopathogens represent a potential new front in non-chemical pest management. Of interest is *Beauveria*

bassiana, reported as an endophyte in a variety of crops, with demonstrated negative activity against insects and plant pathogens, as well as *Metarhizium anisopliae*, reported to beneficially affect plants. We have taken some of the first steps towards pre-planting inoculation to confer endophytic colonization of these entomopathogens in beans and cassava. Pre-planting treatments included (1) floral inoculation, whereby bean flowers are sprayed with conidial suspensions; (2) seed treatments, whereby bean seeds are immersed in conidial suspensions prior to planting; and (3) stake immersion, whereby cassava cuttings (the propagative unit) are immersed in conidial suspensions prior to planting in the field. The effects of endophytic colonization on plant and insect fitness were assessed, as well as fungal entomopathogen isolation from treated plants and from insects exposed to the treated plants.

F-27 Does *Metarhizium anisopliae* influence strawberries in presence of pest and disease?

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Within a framework of a national funded project, different biocontrol strategies against pests and diseases in strawberry were compared. At the JKI Institute first *in vitro* tests with 98 micro-organisms against four soil borne diseases were performed. Five different entomopathogenic fungi and five bacteria were also tested. *Metarhizium anisopliae* (strain Ma43=F52=BIPESCO5) was one of the most promising isolates, after ranking of all antagonists, inhibiting the growth of *Verticillium* spp. and *Phytophthora* spp.. At the JKI greenhouse and field experiments with strawberry plants cv. Honeoye were performed with *V. dahliae* artificially inoculated soil. In greenhouse trials different growth parameters were investigated. In comparison to the control all growth parameters were positively influenced by Ma43. When four different antagonists (*Trichoderma harzianum, T. atroviride, M. anisopliae* and *Bacillus amyloliquefaciens*) were compared, Ma43 showed the best results for leave dry weight, plant height and leaf surface area per plant. In a field trial the plants were visually ranked. 85% of the plants treated with Ma43 were classified with ranking one whereas in all other treatments these were about 50%. Ma43 was also tested for control of *Antonomus rubi* on a organic field by Bioland Beratung GmbH. Ma43 did not reduce the damage on the stalk of the strawberry blossom. However, significant higher yield of grade 1 strawberries and less quantity of deficient fruits was achieved. For all *ad planta* experiments Ma43 was produced in a Prophyta solid state fermenter. Conidia were harvested with a mycoharvester or washed off from the substrate to obtain a sprayable formulation.

F-28 Entomopathogenic fungi for managing the invasive Bagrada bug, *Bagrada hilaris*

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Bagrada bug (*Bagrada hilaris*) is an invasive stink bug in California and parts of Arizona attacking cole crops and various other hosts. Conventional growers have several chemical pesticide options for managing this new pest, but organic growers and home gardeners are looking for non-chemical solutions. Laboratory assays were conducted using commercial formulations of the entomopathogenic fungi, *Beauveria bassiana* (strain GHA), *Metarhizium brunneum* (strain F52), and *Isaria fumosorosea* (strain FE 9901). Preliminary results indicate that *B. bassiana* and *I. fumosorosea* can be effective in controlling Bagrada bug adults. Unlike some other agricultural pests which are mainly a concern in the crop fields or effectively controlled with various management tools, Bagrada bug poses a serious threat to organic farms or home gardens that have limited control options and offers a good opportunity for exploring microbial control. Additional studies will help develop an effective management strategy with a strong microbial control component.

F-29 Sexually Dimorphic Response of *Drosophila melanogaster* to Infection by Two Strains of *Beauveria bassiana*

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Beauveria bassiana infects a wide variety of insects, but mechanisms of possible resistance to this pathogen – or to any fungus – are poorly understood. As part of a larger series of studies on fruit fly susceptibility to disease, we investigated resistance mechanisms to *B. bassiana* in *Drosophila melanogaster* and documented striking sexual dimorphism in resistance to *B. bassiana*. We screened isolates of the fungus against the Canton-S strain of *D. melanogaster* and chose two (ARSEF 8246 and GHA) for further work. A dose response showed strain 8246 to be more virulent than GHA, but mortality among females was significantly higher after inoculation with either strain. We plan further work on this system to explore possible reasons for this difference.

F-30 High co-infection of entomopathogenic fungi from the "Insect bait" method is obtained by plating sections of the infected insects in the culture medium

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The "Insect bait" method for isolation of entomopathogenic fungi from soil consists of using insect species which are highly susceptible to these fungi. After death and sporulation by the fungus, conidia are removed from the surface of the body and transferred to a culture medium. Studies in our laboratory have shown that during co-infection of *Metarhizium anisopliae* and *Beauveria bassiana*, although the two fungi are developed within the host usually only one species sporulates over the cadaver. In this study, we demonstrated that it is possible to recover more than one entomopathogenic fungus from the same insect when the insect body is cut into pieces after death and placed in culture medium. This was performed by inoculating 1.10^6 conidia of *M. anisopliae*, *B. bassiana* and *Isaria fumosorosea* in 80g sterilized soil containing 10 larvae of *Galleria mellonella* and 10 of *Tenebrio molitor*. The fungus was applied alone or combined. Dead larvae were sectioned into head, thorax and abdomen and each part transferred to plates containing PDA medium. In tests of coinfection, *M. anisopliae* was more prevalent than *B. bassiana* or *I. fumosorosea* in *T. molitor*, but the opposite was observed for *G. mellonella*. When *B. bassiana* and *I. fumosorosea* were applied to the soil together, there was a greater recovery of *I. fumosorosea* in *G. mellonella*, but in *T. molitor* both fungi was observed to develop simultaneously. After sectioning the insect' body, although it was possible to recover both fungi in the same insect, the recovery rates were low in all combination, except *B. bassiana* and *I. fumosorosea* from *T. molitor*. With respect to the sections of the body of insects, fungi were isolated from the head, thorax and abdomen when they were inoculated alone or together in the soil.

F-31 STU Are destruxins important for entomopathogenic fungi virulence?

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Destruxins (DTXs) are secondary metabolites produced by many insect pathogenic *Metarhizium* spp. isolates. The DTXs were first described in 1961; and they immediately were assumed to play an important role in virulence of these fungi to host arthropods. Recent publications on the importance of DTXs as a virulence factor differ widely in their conclusions. The present study evaluates *in vitro* production levels of destruxins A, B and E by 20 *Metarhizium* spp. isolates and correlates these data with each isolate's virulence against *Galleria mellonella* and *Tenebrio molitor* larvae. The isolates were grown in submerged liquid culture and destruxin production was determined by gradient RP-HPLC analysis of culture-filtrate extracts. Destruxin A, B and E concentrations were estimated using pure DTX standard for each compound. In the bioassay, *G. mellonella* or *T. molitor* larvae were sprayed with 1×10^5 or 1×10^7 spores mL^{-1} of each fungal isolate. Mortality was recorded from day 1 to day 10 after treatment. The highest *in vitro* DTX production was by 3 isolates (ARSEF 2521, ARSEF 552 and ARSEF 3463) that also were highly virulent against both insect species; nevertheless, there were some isolates that did not produce detectable levels of any destruxins (ARSEF 760 and ARSEF 724) but were highly virulent; and, in fact, had shorter times to death than some moderate producers of DTX in culture filtrates (ARSEF 782 and ARSEF 729). These results indicate that the presence or absence of DTXs A, B and E in culture filtrates does not correlate with levels or speed of insect kill.
Key words: secondary metabolites; insect biocontrol.

F-32 STU Histopathological observation of infection dynamics of *Beauveria bassiana* in adult female *Anopheles stephensi* using Grocott stain

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Mosquito vector control is an important part of controlling infectious diseases. Although the use of chemical insecticides is the mainstay for disease vector control, development of insecticide resistance has been reported. So new vector control approaches are required. In our previous study, *Beauveria bassiana* 60-2 that showed the highest pathogenicity against *Anopheles stephensi* (LT_{50}: 5.8 days) was isolated from wild mosquito. The aim of this study was to histopathologically observe the infection dynamics of *B. bassiana* in Anopheline mosquito. Initially, the mosquitoes were dissected just after tarsomere topical inoculation and all these body parts (proboscis, head, thorax, wing, leg and abdomen) were placed on the entomopathogenic fungi selective medium. As a result, fungal development was observed from not only the legs but also the proboscis. It means that there are two infection routes on this inoculation method to mosquito. Subsequently, paraffin sections were made from mosquitoes which reared for 1 to 7 days after inoculation, and observed fungal invasion and growth in the mosquito haemocoel and/or tissues by using Grocott stain. Dead individuals were detected from 3 days after inoculation, and fungal propagules were observed at head and brain (massive head infection) in all dead individuals. On the other hand, in surviving mosquitoes, a certain degree of head infection without brain (light head infection) was observed. Our results suggested that fungal infection via the proboscis route is important on rapid mosquito death rather than infection route from tarsomere. Furthermore, rapid mosquito death might correlate with infection level to brain invasion.

F-33 STU *Metarhizium* seed treatment improves conidial dispersal via roots and induces infections in root feeding insects

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The following study investigated whether roots of wheat plants grown from seeds inoculated with *Metarhizium* would carry sufficient conidia to cause infection of root-feeding insects. While most *Metarhizium* research is focused on its development as biocontrol agent, recent studies have highlighted beneficial interactions between *Metarhizium* and plant roots. In the following study, pre-germinated wheat seeds were soaked for 1 hour in either a fungal suspension (four isolate were tested, two *M. robertsii* and two *M. brunneum* at two concentrations: 10^7 and 10^8 condia/ml) or a control treatment (ddH_2O) and planted in either a paper-roll setup or in a pot of non-sterile sandy soil. Roots were harvested at 2 weeks for those grown in the paper rolls or at 2 and 4 weeks for those from the pots. Roots were then washed with ddH_2O and placed with *Tenebrio molitor* larvae, which mimicked a pest insect by chewing on the root to obtain moisture. Dead insects were removed regularly. Additionally, for each treatment, the amount of fungal material remaining on the roots was estimated by counting CFUs from a homogenized sample on selective media. Control insects from both the paper roll and soil setups had very low mortality and showed no mycosis, while several of the fungal treatments had significantly higher mortality than the control and resulted in several cases of mycosis; some variation was observed between the isolates. These results demonstrate that *Metarhizium* conidia can disperse and remain associated with roots in the soil and maintain a sufficiently high dose to infect several weeks after planting, potentially protecting the plant from pest insects. This study also highlights the need to improve our understanding of fungus-insect-root interactions.

F-34 STU Interactions of entomopathogenic fungi and other control agents: mechanism and field potential against pine weevil

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Hylobius spp. are major pests of forestry in Northern Europe and Northern America. The large pine weevil, *Hylobius abietis*, develops in the stumps of recently felled coniferous trees; adult weevils emerge and feed on the bark of seedlings causing mortality. It is estimated that without the use of insecticides, costs due to damage to seedlings would be approximately $184 million per annum in Europe. New EU legislation promotes the use of integrated pest management programmes. We are investigating the use of entomopathogenic fungi (EPF) against pine weevils in conjunction with other control agents. The effect of EPF on the immune system of the pine weevil is being tested to identify immunosuppressive potential of different agents. *Galleria mellonella* (wax moths) are used in parallel and if the results prove to be comparable, wax moths may be used as a model for screening potential EPF isolates. This would be advantageous as wax moths are cost effective and readily available. We identified altered haemocyte activity and a change in insect proteome following treatment with EPF. A bioassay to screen for EPF with potential for synergy with other agents such as entomopathogenic nematodes is currently being developed using strains of *Metarhizium anisopliae* and *Beauvaria bassiana*. This assay will subsequently be applied to novel strains. Our results indicate that selected fungal toxins can suppress the insect immune response rendering the insects more

susceptible to other agents. This study will enable the translation of promising laboratory findings into effective biological control in the field.

F-35 STU Efficacy and Persistence of Two Microbial Control Agents of *Xylosandrus germanus*

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First detected in the U.S in 1932, the ambrosia beetle *Xylosandrus germanus* Blandford (Coleoptera: Curculionidae) is a generalist woodborer native to Asia attacking a wide range of host trees. This wide host range is likely facilitated by the broad range of its symbiotic fungus, *Ambrosiella hartigii*. Despite being considered a secondary pest, *X. germanus* also attacks apparently healthy host trees and so is increasingly being recognized as a key pest of nursery trees. The goals of the current research are to 1) determine beetle survival and reproductive success following application of control agents and 2) evaluate persistence of microbial control agents in the environment. We tested two microbial control agents including *Metarhizium brunneum* strain F52 (Met52, Novozymes, Inc.), which directly affects the foundress and thus indirectly affects gallery formation and offspring, and *Trichoderma harzanium* strain T-22 (Rootshield, BioWorks, Inc.), which directly affects the symbiotic fungus and thus indirectly affects the beetles. Efficacy was evaluated by spraying American beech (*Fagus grandifolia)* sapling bolts with the control agents and measuring variables such as foundress survival, gallery length, and offspring survival. Persistence was tested by spraying control agents on beech sapling bolts, placing them in shaded and sunny environments, and taking samples at designated time intervals. Results from this research will show the potential of these entomopathogenic and mycoparasitic fungi for management of *X. germanus* populations.

MC-1 Susceptibility of two Brazilian *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) populations to the *Bacillus thuringiensis* Cry1Ac toxin

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Plutella xylostella larvae are very susceptible to *Bacillus thuringiensis*-based biopesticides but under high pressure of selection the rapid evolution of resistance to Cry toxins can impair the use of this biological control agent. A field population of *P. xylostella* was collected from a cabbage field in Pernambuco State, Brazil. This field had never been sprayed with *Bacillus thuringiensis*-based biopesticides. From this initial population, two populations were propagated: a "susceptible population" (SP) and a "resistant population" (RP). In the latter population, all *P. xylostella* larvae were exposed to *B. thuringiensis* HD1 for 31 generations at 3 x 10^6 spores.mL^{-1}. When the resistant population reached the 32nd generation, the larvae were bioassayed for susceptibility (LC_{50}) to Cry1Ac (a component of the HD1 strain) and compared with the susceptible population. To evaluate susceptibility kale leaf discs were dipped into a toxin suspension for 10 seconds. Seven concentrations were assayed ranging from 0.025 to 2.5 µg.mL^{-1}. After drying, leaf discs were offered to 100 third-instar larvae from the resistant and susceptible populations per concentration. Larval mortality was evaluated after 120 hours. The LC_{50} estimates for the populations were 0.78 and 0.01 µg/mL respectively. After selection pressure in the laboratory for 32 generations, the resistant population was 78 times more resistant to Cry1Ac toxin than the susceptible population. Studies are ongoing to characterize the nature of this resistance. **Keywords:** entomopathogenic bacterium, biological control, diamondback moth

MC-2 *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) populations show variable susceptibility to *Bacillus thuringiensis* Berliner

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The aim of this study was to verify possible differences between the populations of *Plutella xylostella* (PX, PA, PC, and PJ) from different brassica fields in Brazil by using molecular methods and to evaluate the susceptibility and sublethal effects of these populations to *Bacillus thuringiensis* bioinsecticides and strains. We used COI gene sequencing and inter-simple sequence repeat-polymerase chain reaction to analyze the genetic structure of the populations. The populations showed different genetic structures. To evaluate susceptibility and sublethal effects, 10 strains of *B. thuringiensis* and Btt090®, Dipel®, Agree®, and Xentari® were sprayed on kale leaf discs. After drying, the leaf discs were fed to second instar caterpillars of *P. xylostella* from each population. We observed the insects until the death of the adults and evaluated the following parameters: larval and pupal viability and period, leaf consumption, pupal weight, sex ratio, longevity, number of eggs/female/day, and incubation period and egg viability. Products/strains that caused >80% mortality were used to estimate LC_{50} values. Agree®, Dipel®, Xentari®, 49.19A, E47, and HD1 caused 100% mortality in all the populations. Generally, HD1 and Dipel® were the most toxic because of lower LC_{50}. Among the strains that did not cause 100% mortality, E28 and T08.024 negatively affected the biological characteristics of the pest. The populations of *P. xylostella* were variably affected by the products/strains; therefore, the population and area of occurrence of the pest must be considered prior to choosing an appropriate control measure. **Keywords:** entomopathogenic bacterium, biological control, diamondback moth

MC-3 Identification of Brazilian isolates of *Metarhizium anisopliae* sensu lato and a screening of promising fungal isolates for tick control

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Twenty *Metarhizium anisopliae* s.l. isolates were tested against *Rhipicephalus microplus* larvae. Isolates were identified by sequencing partially the elongation factor gene (EF-1α). Isolates were cultivated onto potato dextrose agar at 25ºC and ≥ 80% RH for 15 days. Four different concentrations of fungal suspensions (10^5, 10^6, 10^7, and 10^8 conidia mL^{-1}) were tested against tick larvae, and larval mortality was then assessed. Lethal concentrations (LC_{50} and LC_{90}) were calculated at day 10 after treatment. One isolate was identified as *Metarhizium robertsii*, and the others as *Metarhizium anisopliae* sensu stricto. The *M. anisopliae* s.s. isolates caused larval mortality ranging from 0% to 99.4% at day 5 after treatment; CG 581 (98.7%

larval mortality), CG 43 (97.5% larval mortality) and CG 520 (99.4% larval mortality) were the most virulent isolates when tested at 1×10^8 conidia mL^{-1}. *M. robertsii* CG 192 caused 96.9% mortality at the same conidial concentration. The isolates CG 127 and CG 34 had intermediate virulence (58.9% and 34.4% larval mortality, respectively); while the other tested isolates caused larval mortality inferior to 25%. Lethal concentrations, LC_{50} and LC_{90}, of the most virulent isolates were, respectively: CG581 (1.89×10^7 and 4.0×10^7 conidia mL^{-1}); CG 43 (3.87×10^6 and 1.04×10^7 conidia mL^{-1}); CG 192 (1.5×10^6 and 2.45×10^7 conidia mL^{-1}); and CG 520 (5.13×10^6 and 2.6×10^7 conidia mL^{-1}). The present study identified and selected Brazilian isolates of *Metarhizium* spp. that were virulent for *R. microplus* larvae and may be further investigated for the cattle tick control. **Key-words:** *Metarhizium anisopliae*; biological control.

MC-4 Identification of Brazilian *Metarhizium* sp. isolates and their virulence against *Rhipicephalus microplus*

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Thirty two *Metarhizium* spp. isolates from Brazil were phylogenetically identified (partial sequence of the gene Elongation Factor 1α), and the virulence assessed against engorged females of *Rhipicephalus microplus*. Tick females were obtained from naturally infested bovines and washed for cuticular asepsis. Fungal isolates were cultivated onto potato dextrose agar medium at 25°C and ≥ 80% RH for 15 days. Spores were used for DNA extraction and for preparation of fungal suspensions (1.4×10^8 conidia mL^{-1}). Ten tick females were used for each group in the bioassay. The following biological parameters of tick females were analyzed: egg production index (EPI), nutritional index (NI) and estimated reproduction, used to calculate the control percentage. 71.9% (n=23/32) of the isolates clustered within the *Metarhizium anisopliae* clade, whereas15.6% (n=5/32) clustered within the *Metarhizium brunneum* clade. Two isolates of *M. anisopliae* were the most virulent, CG 37 and CG 420. The egg mass weight was 35.88% reduced by CG 37 and 80.53% reduced by CG 420. The EPI and NI had 62.44% and 52.98% reduction by CG 37, respectively, while CG 420 reduced 92.53% and 89.05% the EPI and NI, respectively. Tick control percentage was 40.52% when females were treated with CG 37, and approximately 90% when ticks were treated with CG 420. In conclusion, CG 37 and CG 420 are considered promising fungal pathogens to control *R. microplus* tick, and are recommended for field trials.
Key-words: *Metarhizium anisopliae*; biological control

MC-5 Evaluation of different oil-based fungal formulations to control ticks

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Environmental conditions such as humidity, temperature and solar irradiation influences the action of entomopathogenic fungi in the field. Oil fungal formulations can protect spores against adverse environmental conditions and increase the virulence of fungal isolates. In the present study, five *Metarhizium anisopliae* sensu lato (s.l.) isolates, formulated in mineral oil or vegetable oil, were tested against *Rhipicephalus microplus* tick females. The isolates CG 112, CG 347, CG 32, CG 148, and CG 629 were formulated in 10% oil (mineral or vegetable). The association between the isolates CG 148 and CG 629, formulated in the vegetable oil or in mineral oil was also evaluated. Tick females were immersed in 1×10^8 conidia mL^{-1} of each fungal oil-based formulation for three minutes and the following tick reproductive parameters were analyzed: egg mass weight, hatching percentage, egg production index and nutrient index. All tested formulations of both vegetable oil and mineral oil, including the isolates CG 148/CG 629 association, changed significantly ($P \leq 0.05$) the biological parameters of *R. microplus* females. Mineral oil fungal formulations caused, however, higher tick control percentage than vegetable fungal oil formulations. The isolates CG 148 and CG 626 individually formulated in mineral oil caused the highest tick control percentages; while the association CG148/CG629 caused no significant differences in ticks' biological parameters when compared with these isolates formulated individually. In conclusion, the association of highly virulent isolates did not increase tick control.
Key-words: formulation, biological control and ticks.

MC-6 *Sitophilus oryzae* (Coleoptera: Curculionidae) susceptibility to *Beauveria bassiana* (Hypocreales: Cordycipitaceae) at laboratory

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Beauveria bassiana toxic activity for *Sitophilus oryzae* adults was evaluated using IBCB 01, IBCB 17 and IBCB 18 isolates. Four replicate with 15 insects adults were used per isolate. The insects were immersed in a solution with 10^9 conidia.mL^{-1} and removed after 10 seconds to in Petri dishes (9.0 cm diameter) with filter paper and corn (*Zea mays* L .). Treatments were kept in a room at 26 ± 1 °C and 14 hours photoperiod and mortality was evaluated daily until 15 days and data were compared by Tukey (5%). All *Beauveria bassiana* isolates were pathogenic to *S. oryzae* adults, however, mortality ranged from 5.01% to 18.33% (IBCB 18), which is not sufficient for LC_{50} estimatives, and these isolates are not promising to *S. oryzae* management. Some reasons may explain this low potential. The fungus infection begins by the contact of reproductive structures with the insect cuticle. The speed at which these structures can penetrate the insect cuticle depends on the isolate, environmental conditions, the cuticle thickness, the density of conidia and the presence of extracellular enzymes responsible for cuticle degradation. Subsequently, the process of germination and penetration are dependent of factors such as nutritional components of the cuticle, chemical reactions and action of mycotoxins. Furthermore, the genetic variability among isolates of entomopathogenic fungi is also closely correlated with variations in virulence mainly due to the different degrees in ability to penetrate the insect cuticle.

MC-7 Entomopathogenic *Lecanicillium* hybrid strain AaF42 as endophyte for Soybean and its infection process to Soybean Cyst Nematode

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Soybean Cyst Nematodes (SCN: *Heterodera glycines*) are plant-parasitic nematodes caused plant diseases and yield losses in range of crops and cost growers worldwide. One potentially useful agent for biological control is using the entomopathogenic- fungus *Lecanicillim* spp. A potential method of enhancing activity of this species against

soybean cyst nematodes is through the protoplast fusion and breeding of hybrid strain. AaF42 is one of these hybrid strains which have great effective ability for control of soybean cyst nematodes. In this study, we observed characteristic of AaF42 in soybean root surface as an epiphyte and root tissue as an endophyte after soil treatment of AaF42. Colonization on soybean root surface and infection ratio to SCN female were revealed by GFP transformation technique. GFP-transformed AaF42 colonized root surface, epidermal cell and penetrated into intercellular space and inside of cell. And GFP-transformed AaF42 colonized SCN female, cyst and egg. These results confirmed our previous data that cyst of SCN could be infected by AaF42 *in planta*

MC-8 Biocontrol of *Bacillus thuringiensis* to Tomato Fusarium Wilt

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Bacillus species as a group offer several advantages over other bacteria for protection against foliar and root pathogens because of their ability to form endospores, and because of the broad-spectrum activity of their antibiotics. The objectives of this work were to determine the ability of strains of *Bacillus thuringiensis* to inhibit *Fusarium oxysporum* f.sp. *lycopersici* (FOL) growth and to evaluate the ability of biocontrol agent for Tomato Fusarium wilt. Antagonistic reaction of five *B. thuringiensis* strains were identified from dual culture assays with FOL race2 (FOL2). Two strains of *B. thuringiensis* (Bt-AS17,Bt-AS18) showed high antagonistic to FOL2. In independent climate chamber studies, soil application of *B. thuringiensis* strains challenge inoculated with FOL2 recorded low Wilting Score, less than non-bacteria treatment pathogen control. The low disease incidence corroborated with tomato growth promotion and antagonistic reaction to FOL2

MC-9 A Better Toolbox to Understand Efficacy of Met52

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Field trials based on insect population counts are a valuable and required tool for the development of a biopesticide. However, it is also a tool that can give highly variable results and little explanation for this variability. To better understand efficacy we need tools that isolate environmental stressors, track and quantify the organism, and assess pathogenicity. Each of these tools has specific utility and limitations. By using these in concert we can more efficiently improve our knowledge base around field efficacy. Ultimately this helps us to accelerate our development and make better grower recommendations.

MC-10 Improving quantification technologies in *Metarhizium anisopliae*

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The current standard for fungal quantification and viability testing requires plating onto agar and scoring viability with either CFU or % germination which can be time consuming and inconsistent. This poster contrasts a number of tracking tools used to quantify spores as well as determine viability in the entomopathogenic fungus *Metarhizium anisopliae* with an emphasis in the practical application for use in industry. The use of viability staining with tools such as flow cytometry and qPCR have become reliable for many purposes but there are a number of inherent problems when working with fungal spores that can affect results. Refining the quantification methods will help to reliably cut down the time to test product efficacy for *Metarhizium* and can be applied to other products in the future.

MC-11 Antimicrobial activity of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae)

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The black soldier fly, *Hermetia illucens* is commonly inhabit manure and food waste. This study was carried out to identify the antibacterial activity of the extracts of whole black solider fly larvae. The extracts were prepared by homogenizing after mixing with grinded larvae plus 0.01% acetic acid at 4°C for 12hr. Two methods, agar well diffusion and growth curve assay were used to study the antibacterial activity of pathogens. In our studies, the extracts showed antibacterial property as growth inhibition in some tested bacterial pathogens. The data provide further evidence that larvae extracts play a role in the defense against microorganisms.

MC-12 Compatibility between pesticides and *Bacillus thuringiensis* (Berliner)

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The use of entomopathogenic bacterium *Bacillus thuringiensis* as biopesticides or transgenic plants is highy efficient against lepidopteran pests, but in some instances pesticides plus *Bacillis thuringiensis*-based biopesticides are necessary, mainly in crops with a great number of target pests, e.g cotton. This research aimed to analyze the compatibility of pesticides and a *B. thuringiensis kurstaki* based biopesticide, recommended in cotton, using the commercial biological product Dipel® SC, at the minimum concentration (0.50 L. ha^{-1}), recommended for the control of *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) and the pesticides Oberon Plant® at 0.50 L. ha^{-1} ,Talstar® 100EC at 0.55 L. ha^{-1}, and Talisman® at 1.00 L.ha^{-1}. Nutrient agar (NA) plus pesticide was prepared as the microorganism growth substrate and dropped into seven petri plates (replicates). The control consisted only by the culture medium without pesticides. After solidification of the medium it was inoculated 5.0 uL of *B. thuringiensis* biopesticide. Colony growth was measured every 24 hours over five days, and the data compared by Tukey test at 5% probability. The pesticide Oberon®, provided *B. thuringiensis* colonies reaching 59.61 cm^2 in 120 hours and did not influence negatively the bacterium vegetative growth, otherwise the pesticides Talstar® 100EC, provided colonies reaching 9,76 cm^2 in 120 hours, and Talisman®, with no colonies growth, interfered significantly in the bacterial growth. Pesticides and *B. thuringiensis* biopesticides can be used together against *H. virescens*, but it's essential to study the compatibility between them, to assure the pest control.

MC-13 Intraguild interaction between the entomopathogenic fungi *Beauveria bassiana*, the parasitoid *Diaeretiella rapae* and the host *Myzus persicae*

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The intraguild interaction between the entomopathogenic fungi *Beauveria bassiana* and the parasitoid *Diaeretiella rapae* and the host *Myzus persicae* was evaluated under laboratory conditions. Nymphs of *M. persicae* were sprayed with a solution of *B. bassiana* based biopesticide conidia added with Tween® 80 (0.05%), 0, 24 and 48 hour before and after to parasitism by *D. rapae*. The evaluated parameters were percentage of mummies, sex ration, F1 emergence, longevity, period of oviposition until mummification and period of oviposition until emergence. The reduction of the percentage of produced mumies by females of *D. rapae* was significantly influenced by the previous spraying with the entomopathogen. The decrease of the parasitoid emergence percentage and the female longevity of the F1 generation were observed in the treatments with *B. bassiana*. The development period of *D. rapae* was negatively influenced by *B. bassiana*. The negative effects observed between the interaction with the entomopathogenic fungi *B. bassiana* and the parasitoid *D. rapae* demonstrates the constrains of this combination when used for a *M. persicae* biological control program.

MC-14 The effect of the entomopathogenic fungus *Lecanicillium longisporum* (Petch) Zare & Gams (Hypocreales: Cordycipitacea) on the aphid parasitoid *Diaeretiella rapae* McIntoch (Hymenoptera: Braconidae)

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The effect of the entomopathogenic fungus *Lecanicillium longisporum* on the parasitoid *Diaeretiella rapae* was evaluated under laboratorial conditions. Nymphs of *M. persicae* were sprayed with autoclaved distilled water mixed with Tween® 80 (0.05%) (control) or a solution of the biological formulated product containing *L. longisporum* conidia mixed with the adhesive spreader Tween® 80 (0.05%), performed for 0, 24, or 48 h before or after parasitism by *D. rapae*. The evaluated parameters were percentage of mummies, sex ration, F1 emergence, longevity, period of oviposition until mummification and period of oviposition until emergence.The percentage of parasitism was reduced from 66.5 to 17.8% by previous spraying with the entomopathogen on *M. persicae*. The parasitoid emergence and female longevity, offspring of the F1 generation, were significantly affected by *L. longisporum*. The entomopathogenic fungus was prejudicial in the development of *D. rapae* after the initial exposure of *M. persicae* to parasitism. The negative effects from the interaction of the entomopathogenic fungus *L. longisporum* and the parasitoid *D. rapae* demonstrate the problem when used together in the biological control of *M. persicae*.

MC-15 Pathogenicity to *Tenebrio* larvae of *Metarhizium* spp. isolates cultured from Western United States soil

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Metarhizium spp. fungal isolates vary greatly in their ability to infect host insects. Many factors must be considered when screening fungi for their potential as effective biological control agents, including their virulence to insects. The following study surveys 683 *Metarhizium* isolates for virulence toward *Tenebrio molitor* larvae. All isolates were cultured from Western U.S.A. soil in 2011 and 2012, and have been deposited in the ARSEF culture collection, Ithaca, NY. For insect assays, conidia were suspended in 0.01% Tween 80® at 10^5 and 10^7 conidia mL^{-1}. Each *Tenebrio* larva was treated individually with 5 µl of fungal suspension and incubated at 28°C for 8 days, with survival checked daily. The 683 new isolates were tested in 51 groups, each group consisting of about 14 isolates in addition to control (no fungus) and standard isolates [ARSEF 2575 (*M. robertsii*) and ARSEF 1095 (*M. brunneum*)]. The average untreated-control mortality on day 4 and 8 was ~ 4 % and ~ 11 %, respectively. With ARSEF 2575, day 4 mortality at low dose was ~ 18 %, and high dose ~ 62 %, and at day 8 low dose ~ 87 %, high dose ~ 96 %; whereas with ARSEF 1095 day 4 low dose ~ 7 %, high dose ~ 20 %, day 8 low dose ~ 80 %, high dose ~ 90 %. This 2-dose, survey-type assay revealed that, based on time to kill, the new isolates varied widely in their effectiveness versus *Tenebrio*. Approximately 50 % were as effective as the standard isolates. Several of the isolates have been or will be tested versus grasshoppers/Mormon crickets.

Key words: entomopathogenic fungi, mealworms, pathogenicity, biological control.

MC-16 Comparative transcriptome analyses for the invasive insect pest, Brown Marmorated Stink Bug (*Halyomorpha halys*) gene profiling identifies new associated microbes.

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An effective, environmentally sound means for stanching the aggressive North American expansion of *Halyomorpha halys*, the brown marmorated stink bug (BMSB), is needed for protecting a wide array of specialty and staple crops. Microbial biocontrol approaches offer an important possibility, and RNA-Seq was conducted to facilitate gene discovery and to identify BMSB gene targets for molecular biopesticides. RNA-seq libraries derived from whole BMSB insects representing developmental stages and sexes—2^{nd}-instar nymphs, 4^{th}-instar nymphs, adult males and adult females—were sequenced on an Illumina HiSeq 1000. Reads surviving quality control were globally assembled using the Trinity RNA-Seq assembler, yielding 248,569 putatively unique transcripts (PUTs). These were segmented into three disjoint tiers of varying reliability—gold (4,794 PUTs), silver (16,878) and bronze (14,357)—on the basis of alignments with proteins from NCBI NR; PUTs were further annotated using protein family (Pfam) and gene ontology (GO) annotations associated with best NR hits. Blat alignment of cleaned, unassembled reads to gold-tier PUT templates enabled accumulation of sample-specific digital gene expression data, and observed fold changes in gene expression suggested interesting differential gene expression patterns. The presence of novel microbes was recognized; a new BMSB virus is the subject of a concurrent presentation (Sparks et al.). Over 20 PUTs similar to hypothetical proteins of *Nosema ceranae* were more abundantly expressed in adults versus nymphs, as were PUTs encoding ribosomal and histone proteins homologous to those of *N. bombycis*. The surveyed BMSB adults harbor a potentially novel *Nosema* species currently under investigation for pathogenicity to pentatomidae.

MC-17 A comparison of fungal band formulations for Asian longhorned beetle biological control

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Experiments were conducted with the entomopathogenic fungus *Metarhizium brunneum* to compare the efficacy of agar-based fungal

bands versus two new types of oil-formulated fungal bands for the management of Asian longhorned beetles. We monitored conidial retention and survival on three types of fungal bands attached to trees in New York and Pennsylvania. The band types were a standard polyester fiber agar-based bands containing fungal cultures, and two types of bands that were made by soaking either polyester fiber or jute burlap with oil-conidia suspensions. Fungal band formulation had no effect on the number or viability of conidia on bands over the two-month test period, although the percentage of conidia that remained viable decreased significantly over time for all band types. In a laboratory experiment testing the effect of the three band formulations on conidial acquisition and beetle survival, standard agar-based fungal bands delivered the most conidia to adult beetles and killed higher percentages of beetles significantly faster (median survival time of 27 d) than the two oil-formulated materials (36-37 d). We also tested the effect of band formulation on conidial acquisition by adult beetles kept individually in cages with a single band for 24 h, and significantly more conidia (3-7 times) were acquired by beetles from agar-based bands compared to the two oil formulations.

MC-18 Effect of *Bt* bioinsecticides on the life history of *Podisus nigrispinus* over generations

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The impact of *Bt* bioinsecticides on the developmental rate, life history, and fertility of the predator *Podisus nigrispinus* was examined over 3 consecutive generations. The predators were fed on larvae of alternative prey *Diatraea saccharalis* and a suspension of *Bt* bioinsecticides as the source of water. The following 3 treatments were used: suspension of *Bacillus thuringiensis kurstaki* HD1; suspension of a commercial product containing *B. thuringiensis* subsp. *aizawai* CG 91 (Agree®); and water (control). The results showed that the net reproduction rate (R_0) of *P. nigrispinus* that received the control treatment was higher in the first generation; however, the R_0 of *P. nigrispinus* that received the Agree® treatment was similar to that of the control in the third generation. The intrinsic rate of increase (r_m) was similar in the first and second generations; however, in the third generation, *P. nigrispinus* that received the control and Agree® treatments showed higher values. The mean generation time (*T*) was low to the Agree® and control treatments. In the third generation, the doubling time (*D*t) of *P. nigrispinus* that received the control and Agree® treatments was lower than the *D*t of those that received the HD1 treatment. These results indicate that Agree® did not affect the fecundity and survival of *P. nigrispinus*. Thus, Agree® and *P. nigrispinus* can be used in conjunction and contribute to maintaining the balance of the agricultural ecosystem and food production that is safe for human consumption.**Key words**: Asopinae, insect biology, biological control, microbial control

MC-19 Compatibility of Bt-based biopesticides and chemical pesticides recommended for *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Increasing reports of pesticides resistance in tomato pests populations, as the leafminer *Tuta absoluta* are threatening the sustainable use of control tatics. Bt-based biopesticides are an important tool to manage the tomato leafminer populations resistance. However, up to now few studies were carried out evaluating the interactions between chemical pesticides and Bt-based biopesticides. The compatibility of chemical pesticides (azadirachtin, flubendiamide, bifenthrin, spinosad, and beta-cyfluthrin) recommend for tomato leafminer and a Bt-based biopesticide (Dipel) was measured. The evaluated parameter was the colony size, which is directly associated with growth of the bacterium. The higher and lower dose of each chemical pesticide were mixed into the culture medium when the temperature reach to 45ºC. As soon as the medium solidified 5µL of the bioinsecticide was inoculate in the center of the plate and spread in the whole plate with a handle. Ten replications were used per treatment and in the control was inoculated Bt-based biopesticide in culture medium with no chemical pesticide. The evaluation was performed each 24 hours until 168 hours measuring the area of *Bacillus* colony. Azadirachtin, flubendiamide, bifenthrin, spinosad, provided the higher growth of bacterial colonies and were similar to control. The pyrethroid pesticide beta-cyfluthrin provided lower growth of colonies. Furthermore, pesticides applied at a minimum dose provided larger colonies. The compatibility between Bt biopesticide and chemical pesticides depends on the pesticide itself and the concentration assayed.
Key words: insect biology, biological control, microbial control

MC-20 Pathogenicity of *Beauveria bassiana* microencapsulated in dry and wet formulations against *Diatraea sacharalis*

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The sugarcane borer (*Diatraea saccharalis*) is the most important pest in the cultivation of this crop. The use of entomopathogenic fungi is being used in sugarcane, but without an efficient formulation in Brazil. The objective of this study was to evaluate in laboratory encapsulated formulations, dry and wet, with *Beauveria bassiana*, against this pest. It was used IBCB 66 (B. bassiana) isolated formulated in alginate capsules. The capsules of sodium alginate were used wet or dry. The dry formulation was obtained upon drying for 4 days in an incubator at 25 ºC, being the original formulation wet. In each pot containing 6 caterpillars was added 0.05 g of the formulations and the control was done with 0.05 g of pure conidia. A control was also performed with water only. As expected the pure conidia showed higher mortality of caterpillars in less time, 100% after 7 days. Already, the dry and wet formulations provided mortality at 7 days from 40.2 and 29.6% and at 15 days of 79.6 and 66.8%, respectively.

MC-21 Tolerance of conidia formulated and unformulated of *Metarhizium anisopliae* at UV radiation

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The formulation of entomopathogenic fungi is one of the major obstacles hampering the expansion of the use of microbial control in the world and your success. The unformulated fungi are sensitive to radiation hindering mainly their application. Thus, the objective of this study was to evaluate the effects of UV radiation to formulated and non formulated *Metarhizium anisopliae* conidia. The pure conidia and the formulated conidia in sodium alginate (IBCB 425 isolate) were maintained in a open Petri dishes and were placed in a chamber irradiated by actinic four lamps Philips 15 W whose emission spectrum is concentrated in the region of UVA/UVB under controlled temperature of 25 ± 2 °C. A control was done with the Petri dishes completely covered with aluminum foil. Samples were withdrawn during 2, 4, 6, 8, 10, 12 and 24 hours and analised for viability of conidia which 0.05 g was mixed in 100 mL of sterile distilled water

with Tween ® 80. An aliquot of 0.1 mL was plated on Petri dishes containing a thin layer of BDA with pentabiotic (0.5 g/L). The plates were incubated at 25 ± 0.5 ° C for 24 hours and after this time the examination was done by optical microscopy to count the spores germinated and not germinated, establishing a ratio. The sodium alginate formulation protected the conidia that lost less viability than the unformulated fungi. After 24 h of irradiation the conidial unformulated showed 24% germination while formulated had 62%; controls both conidia remained with viabilities above 80%.

MC-22 Compatibility of Met 52 with key augmentative biological control insects

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Metarhizium anisopliae (specifically in Novozymes BioAg formulations Met 52 and Met EC) is an entomopathogenic fungi gaining use in integrated pest management (IPM) programs. As the use of *Metarhizium* increases it has become incumbent to understand how it interacts with key biological control insect pests. Several important augmentative biocontrol insects were screened to determine I.O.B.C. (International Organization for Biological and Integrated Control of noxious animals and plants) classifications of Met in relation to the beneficial insects. Direct toxicity tests were conducted with *Amblyseius swirskii, Encarsia formosa, Eretmocerus spp*., *Macrolophus caliginosus, Nesidiocoris tenuis,* and *Phytoseiulus spp*. This data will lead to development of integrated programs utilizing both entomopathogens and augmentative beneficial insects.

M-1 Mobility of gypsy moth larvae infected with the microsporidium *Nosema lymantriae*

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The gypsy moth, *Lymantria dispar* L. (Lepidoptera, Lymantriinae) is host for a variety of several microsporidian species. *Nosema lymantriae* is a systemic microsporidian pathogen of *L. dispar* that is efficiently transmitted among gypsy moth larvae in small scale experiments. The spatial spread of pathogens is an important determinant for epizootics in structured host populations. Newly molted third instar test larvae and larvae inoculated with *N. lymantriae* 13 and 19 days before were allowed to feed together and to move freely on and between potted oak plants arranged in one line for a period of three days. The mobility of inoculated larvae reduced with the progression of infection. On average, 55.7% and 38.4% of the inoculated larvae moved between the trees, when they were still in the latent phase or in the infectious phase of the microsporidian infection. No differences were recorded in the mobility of test larvae infected or not infected with *N. lymantriae*. A higher proportion of test larvae stayed at the same potted oak plant when the distance between the oak plants increased; 64.4% and 46.7% of the test larvae were recovered at different plants when the distance between the plants was 0.5 m and 2 m.

M-2 Characterization of a new microsporidium parasite of *Dendroctonus ponderosae* (Coleoptera, Curculionidae, Scolytidae).

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The mountain pine beetle (MPB) (*Dendroctonus ponderosae*) is a native bark beetle of western North America that attacks pine tree species, particularly lodgepole pine.

The beetle's lifestyle is mostly cryptic and no insecticides are known that would efficiently target all life stages under the bark. Therefore, there is an urgent need to search for adequate natural enemies that could be used as alternatives to manage MPB. A new microsporidium species was isolated and characterized from MPB adults. The infection was restricted mostly to the gut epithelial cells. Ultrastructural features indicated that the new microsporidium possesses dichotomous key of the genus *Nosema*: binucleate meronts, sporonts, sporoblast and develop in direct contact with the host cell cytoplasm. Mature spores are diplokaryotic, measured 2–3 × 1-2 µm in fixed spores and have isofilar polar filament with 6-7 coils. There was at most a 99% nucleotide sequence identities in the SSU rRNA gene between the new isolate designated here as *Nosema* sp. MPB and other *Nosema* species. The SSU rRNA phylogeny also placed *Nosema* sp. MPB in the *Nosema* cluster which further confirmed the classification of the new isolate. The organization of ribosomal RNA gene sequence of *Nosema* sp. MPB show a 5'-LSU rRNA-ITS-SSU rRNA-IGS-5S-3' arrangement which is an important feature of the ''true'' *Nosema* group. Prevalence was very low (15 %, n=120) for the three years sampled and no sex dependent differences in infection could be observed. Implications for the infection of MBP populations by *Nosema* sp. MPB will be discussed.

M-4 STU Description of *Hepatospora* sp. infecting Pea crab (*Pinnotheres pisum*) – a commensal of marine mussels (Mytilus spp.)

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The pea crab (*Pinnotheres pisum*) is considered a commensal organism living amongst the soft tissues of marine mussels (*Mytilus* spp.) and other bivalve molluscs. Here we describe a microsporidian pathogen within the hepatopancreatic tubules of *P. pisum* found within mussels collected in the United Kingdom. The pathogen occurred within an interfacial membrane within the cytoplasm of hepatopancreatic epithelial cells and elicited tubular degeneration during advanced infection. Histologically, the disease caused by the microsporidian was indistinguishable from that observed in Chinese mitten crab (*Eriocheir sinensis)* infected by *Hepatospora eriocheir* (Stentiford et al., 2011). However, ultrastructurally, the pathogen differed significantly by displaying a diplokaryotic status throughout all observed stages of its life cycle. Sequencing of the partial SSU rRNA gene confirmed the close relationship (99% similarity) between the pea crab parasite and *H. eriocheir*, and to another *Hepatospora* sp. described infecting the European edible crab (*Cancer pagurus*). This description provides a further example of a gut-infecting microsporidian pathogen residing within the family Hepatosporidae and suggests that the family forms a growing clade with members of the family Enterocytozoonidae. The latter also contains parasites infecting the gut of marine crustaceans but also parasites of aquatic vertebrates (fish) and humans. The placement within the Hepatospora genus (despite ultrastructural distinctions to the type species *H. eriocheir*) takes in to account the potential for plasticity in closely related microsporidian taxa and supports the potential for closely related organisms with the *Hepatospora-Enterospora* clade to exist in both a wide range of ecological niches and in a divergent range of host taxa.

N-1 Correlation study of genetic profiles associated with differential phenotypes of populations of the nematode *Strelkovimermis spiculatus* parasite of mosquitoes of public health significance

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Mosquitoes are public health importance insects which can be controlled by natural enemies such as nematodes. In that framework, *Strelkovimermis spiculatus* Poinar and Camino, 1986 (Mermithidae), was found in temporary flooded areas from larvae of the mosquito *Ochlerotatus albifasciatus* and later from *Culex pipiens* larvae in permanent flooded sites, both in Argentina. Actually, molecular biology is used to distinguish among closely related species of nematodes focusing in the sequence analyses of mtDNA and rDNA *loci*. In view of that, the aim of this study was determine whether various populations of putative *S. spiculatus* (collected from La Plata, Argentina) had molecular differences that resulted in changes in their bio-ecological characteristics. Thus, the *18S* ribosomal nuclear DNA, and *cox1* and *nd4* mitochondrial genes were evaluated by PCR and subsequent sequencing using standard and *ad hoc* designed primers. Bioinformatics analyses revealed that the different populations tested had various polymorphisms but all nematodes belonged to the same species. The genetic intraspecific variations observed could explain the different grade of parasitism previously assayed and suggest the existence of different natural strains. In consequence, the strategy applied allows establishing the first experimental approaches to detect and use genetic biomarkers in *S. spiculatus* assignable to phenotype properties. This information will be used in future to assist in the characterization of the best wild type population of nematodes in terms of their natural capability to infect mosquitoes.

N-2 Pheromones regulate nematode dispersal

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Dispersal is an important nematode behavior for survival. Upon crowding or food depletion, the free living bacteriovorus nematode *Caenorhabditis elegans* produces stress resistant dispersal larvae, known as dauer. Other nematodes also have dispersal larvae. In plant parasitic *Meloidogyne spp*. it is called J2 and in insect parasites (entomopathogenic nematodes, EPN) it is known as infective juveniles (IJs). Even though pheromones regulate entry into dispersal larvae in *C. elegans* and insect parasites, it is not known whether pheromones regulate dispersal. We hypothesized that pheromones may regulate dispersal behavior in *C. elegans* and in other nematodes. Liquid chromatography-mass spectrometry analysis of *C. elegans* dauer/dispersal supernatant with dispersal activity revealed four known ascarosides (ascr#2, ascr#3, ascr#8, icas#9). A synthetic pheromone blend at physiologically relevant concentrations dispersed *C. elegans* in the presence of food and also caused dispersion in insect parasite (*Steinernema feltiae*) and plant parasitic (*Meloidogyne spp*). Assay guided fractionation revealed structural analogs as major active components of the *S. feltiae* (ascr#9) and *C. elegans* (ascr#2) dispersal blends. Further analysis revealed that all *Steinernema* spp. and *Heterorhabditis* spp. infected insect host cadavers share a common pheromone, ascr#9, suggesting one species can recognize another's blend. Pheromones are fundamentally important for nematode communication across diverse habitats, and thus may provide sustainable means for control of parasitic nematodes.
We thank NemaSym for the travel support.

N-3 Evaluation of *Xenorhabdus bovienii* or *Photorhabdus luminescens* bacterial supernatant against the root-knot nematode *Meloidogyne incognita* (Tylenchida: Meloidoginidae)

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The root-knot nematode (RKN), *Meloidogyne incognita* a major pest of vegetables in many parts of the world, causes poor growth, a decline in crop quality and yield, and reduced resistance to other stresses. This RKN causes significant damage to greenhouse vegetables in the coastal regions of Turkey. Previous studies demonstrated that the mutualistic bacteria of entomopathogenic nematodes had adverse effects on RKN populations and reduced damages to plants. Our objective was to determine the effects of the bacterial supernatant of *Xenorhabdus bovienii* (associated with *Steinernema feltiae*) or *Photorhabdus luminescens* (associated with *Heterorhabditis bacteriophora*) on the Turkish isolate of *M. incognita* race 2 in the greenhouse. Experimental units were plastic pots (7×7×7 cm) containing sterilized loamy sand and one 20-day-old tomato seedling (SC-2121 variety). Three thousand *M. incognita* eggs were applied at 2 cm soil depth. Two treatment methods were used. These were (A) 10 ml of 7-day-old bacterial supernatant (*X. bovienii* or *P. luminescens*) in TSB medium applied with a syringe into the soil, and (B) dipping the tomato seedling root in the bacterial supernatant before transplanting in the infested pot. The two controls were pots with seedlings with (A) 3000 RKN eggs, and (B) no RKN eggs. Bioassays were conducted in October and December 2012. The experiments contained five replicates (pots) for each treatment. The following data were recorded 9-weeks post treatment: (1) total number of egg masses per plant, (2) plant height, (3) fresh and dry root weight, and (4) fresh and dry weight of stems and leaves. The results showed that both treatment methods of *X. bovienii* supernatant significantly suppressed the effects of RKN on the plant. Immersion of tomato roots pre-transplant in the *X. bovienii* supernatant was more effective than the syringe method.

N-4 Virulence of entomopathogenic nematodes against the oil palm borer, *Eupalamides cyparissias cyparissias* (Lepidoptera: Castniidae)*

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The oil palm borer, *Eupalamides cyparissias cyparissias*, is an important pest of oil palm (*Elaeis guineensis)* plantation in Brazil and other countries in South America. Early instars feed on the surface of the petiole, covered with plant debris, and then perforate the stipe. They create sinuous tunnels with irregular borders. The virulence of entomopathogenic nematodes was evaluated against 9-11° instar larvae of *E. cyparissias* at laboratory and field conditions. For the laboratory test, three treatments were established: *H. indica*, *S. brazilense* and control. For each treatment, 15 replications were used, each replication represented by a larva. The nematodes were tested at the doses of 12,000 IJ/insect. A field test was accomplished in an oil palm plantation, in Tailândia municipally, PA, Brazil. Three treatments were established: *S. brazilense* applied at the dose of 1,000,000 IJ/plant (1 x 10^8 IJ/ha) using the volumes of 12 L suspension/plant; and 2,000,000 IJ/plant using the volume of 24 L of suspension/plant (2 x 10^8 IJ/ha); and control. For each treatment, 3

replications were used, each replication represented by an oil palm plant (8-10 m height). The nematode was applied on the top of the plants using a pressurized hose. Previously to the application, larvae were held in the insertion of stem, next to the stipe. Both nematodes were pathogenic to the larva, with *S. brazilense* being the most virulent and killing 93% of larvae in 23 days. In the field, *S. brazilense* caused > 90% mortality of larvae at the higher dose, 2 months after the application.

N-5 Heme-responsive genes in *Brugia malayi* and its obligate endosymbiont, *wolbachia*

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Brugia malayi (*Bm*) is one species of parasitic nematode that causes lymphatic filariasis, a debilitating disease affecting nearly 150 million people worldwide. *Bm* contain *Wolbachia* (*wBm*), an obligate intracellular endosymbic bacterium. Interestingly, genome sequencing of *Bm* and *wBm*, identified a number of critical metabolites implicated in the host-endosymbiont interaction, one of which was heme. By serving as a co-factor in a number of enzymes, heme is essential to many biological processes. Although *Bm* contains a functional ferrochelatase gene (the final step in the heme biosynthetic pathway and a product of lateral gene transfer), like other nematodes they are incapable of synthesizing heme. However, the *wBm* genome contains a nearly complete and likely functional heme synthesis pathway, leading to the hypothesis that *wBm* may supply *Bm* with heme. A better understanding of how *Wolbachia* contributes to overall *Bm* fitness, possibly through the critical enzymatic co-factor heme, is essential to the development of treatments with enhanced targeting of both *Bm* and *wBm*. Our laboratory has exploited the use of next generation sequencing to investigate differential expression patterns of *Bm* in response to heme, The preparation of samples using the NEBNext mRNA Library Prep Master Mix Set for Illumina allows us to utilize total RNA extracted from *Bm*. Interestingly, many genes from both *Bm* and *wBm* are heme-responsive (differentially expressed in the presence of heme). These observed heme-responsive genes in both *Bm* and *wBm* provide critical insight into the obligate endosymbiotic relationship between the two organisms.

N-6 *Steinernema* chemotaxis to cognate and non-cognate symbionts

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A few studies suggest *Steinernema* nematodes have varying degrees of specialization for their symbionts. It has been shown that non-cognate *Xenorhabdus* spp. can have adverse effects on nematode fitness. *X. bovienii* is the bacterial symbiont with the broadest host range known. At present, nine nematode species belonging to two different evolutionary clades harbour this bacterium. It is unknown if nematode hosts are specialized for this bacterial species or for specific strains of this species. In this study, we explored attraction activity of three *X. bovienii* hosts: *S. puntauvense*, *S.oregonense* and *S. feltiae*. Four *X. bovienii* (puntauvense, feltiae-FL, oregonense, intermedium) and one *X. nematophila* strains were used. A lipid agar-plate bioassay was developed to determine nematode attraction for (1) cognate and (2) non-cognate symbionts. On each plate, 50 µL drops of 100 IJs were placed at the center and bacterial choices 1 and 2 were inoculated 2.7 cm from the center. Plates were placed on a tray in the dark at random positions and incubated at room temperature for 3 days. Nematodes that moved to each target zone on the plate were counted. An Attraction Activity Index (AAI) was considered for measuring attraction of *Steinernema* species to cognate and non-cognate symbionts. Results indicate that all *Steinernema* spp. tested preferred their cognate symbiont and repelled bacterial symbionts that were of a different species. Additionally, nematodes exposed to non-cognate symbionts of the same species (different strain), showed various degrees of attraction. However, AAI values were lower than that observed for cognate symbionts.

N-7 Entomopathogenic nematodes from *Steinernema intermedium* group – Molecular analysis suggests existence of more than five species.

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Nematodes are considered one of the most difficult animals to identify. In the past, the identification of entomopathogenic nematodes was based mainly on morphology and morphometry of adult males and infective juveniles. In the last twenty years, DNA based diagnostic methods have been developed to support traditional identification. Presently, morphological and molecular data together make a robust identification tool that has changed the view on nematodes taxonomy, but also facilitates a discovery of new species. *Steinernema intermedium* group consists of four species up to these days: *S. intermedium*, *S. affine*, *S. sichuanense*, and *S. beddingi*. Recently one new (fifth) species belonging to this group was recovered from South Bohemia, Czech Republic. This species is very well supported both morphologicaly and molecularly, but it was probably misidentified with *S. intermedium* in the past. Medium body length of infective juveniles is 898 µm. For first generation males, the diagnostic characters include the spicule length of 73 µm. Both generations possess characteristic protuberances or irregularities at the tip of the proximal part of the gubernaculum. Females have an indistinct, symmetrical, rarely slightly asymmetrical vulva. The tail of mature first generation females is obese with a short conical tip (peg) with one minute protuberance and a postanal swelling is not developed. Consequent analysis of molecular data gained from sequencing of 28S and ITS regions of this group together with data available in GenBank suggests that that Intermedium group probably contains other, as yet undescribed, species.

N-8 Can entomopathogenic nematodes carry conidia below ground?

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Several studies investigating the combined use of fungi + entomopathogenic nematodes have reported increasing insect mortality. In the current study, IJs of *Steinernema brazilense* IBCB n6 were evaluated concerning their capability to carry conidia of *Beauveria bassiana* IBCB 170 and *Metarhizium anisopliae* IBCB 383. Treatments were: 1) *S. brazilense*; 2) *B. bassiana*; 3) *M. anisopliae*; 4) *S. brazilense* + *B. bassiana*; 5) *S. brazilense* + *M. anisopliae;* 6) Control (water). Five replicates were used for each treatment, each replicate composed by a plastic pot (500 mL) containing sandy soil moistened to 10%. In the bottom of the pot, three *Galleria mellonella* larvae were trapped aiming to attract the nematodes. All treatments were applied over the soil using 3 mL of suspensions/pot, at concentration of 3 IJ/cm^2 for nematode, and 10^8 con. mL^{-1} for the fungi. Evaluation was done seven days after the application, taking soil samples (1g) at 0-4 cm, 4-7 cm, and 7-10 cm deep in the soil to evaluate the conidia concentration. For all treatments with *M. anisopliae*, conidia were found only on the superficial stratum (301 CFU). For the treatment with *B. bassiana* alone, colonies were found mostly in the superficial layer: 125,33 CFU in 0-4 cm, 0,00 CFU in 4-7 cm and 31,00 CFU in 7-10 cm deep. However, for the treatment *B. bassiana* + *S. brazilense*, the number of

CFU increased significantly in the deepest stratum: 142 CFU in 0-4 cm, 24,67 in 4-7 cm and 469,00 CFU in 7-10 cm deep.

N-9 Virulence of entomopathogenic nematodes to larvae of the guava weevil, *Conotrachelus psidii*

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The guava weevil, *Conotrachelus psidii*, is the main pest of guava in Brazil, causing extensive damages to the fruits. The larvae grow inside the fruit until reaching the fourth-instar, when they leave the fruit and migrate down to the soil. In the soil, the larvae burrow to some deep, remaining underground as pre-pupa stage for about four month, till the pupation. Entomopathogenic nematodes (EPNs) are potential candidates as biocontrol agent of these larvae. The current study evaluated several species of EPNs against pre-pupae stage of *C. psidii*. Thirteen isolates from eight EPN species were tested: *Heterorhabditis amazonensis* isolates AM 124 and AM 71; *H. indica* IBCBn 05; *Steinernema carpocapsae* IBCBn 02; *S. brazilense* IBCBn 06; *S. feltiae* IBCBn 47; *S. puertoricense* CER 125; *S. rarum* PAM 10, PAM 29 and PAM 42 and *Steinernema* sp. AM 39, AM 132 and CER 17. The larvae were gathered in pots containing sandy soil moistened to 10%. Five larvae were used per pot and five replications per treatment. The concentration was standardized in 500 IJ/insect, using 10 mL of suspension per pot. Evaluation was done 10 days after application. *H. amazonensis* AM 124 was the most virulent isolate, causing 84% of confirmed mortality, followed by *H. amazonensis* AM 71 (36%) and *S. puertoricense* CER 125 (32%). These results showed that *H. amazonensis* AM 124 is a potential as biocontrol agent of pre-pupae of guava weevil.

N-10 *Steinernema glaseri* infection decisions change when exposed to potential hosts infected with entomopathogenic fungi

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Entomopathogenic nematodes are exposed to a range of potential hosts in the soil. It is likely that IJs encounter hosts that have been exposed to other pathogens such as entomopathogenic fungi (EPF). The purpose of these assays was to assess the infection decisions made by entomopathogenic nematodes (*Steinernema* spp.) in host choice assays, focusing on hosts that had been previously exposed to an entomopathogenic fungus (*Metharhizium anisopilae*). In each assay, 50 IJs were exposed in sand arenas to two waxworms: either two previously uninfected waxworms, one uninfected and one *M. anisopilae* exposed, or two *M. anisopilae* exposed. We also assessed the effect of post-EPF exposure duration by using hosts that had been exposed to *M. anisopilae* either 24, 48, or 72 hours prior to their addition to the experimental arenas. *S. glaseri* IJs preferentially infected fungal-infected waxworms compared to uninfected (average ratio of 2.3 to 1, respectively). *S. glaseri* IJs in general invaded fungal-infected hosts in greater numbers than uninfected hosts; the time after fungal exposure did not appear to influence this preference. The positive response to fungal-infected hosts may be due to short-term increases in carbon dioxide production from fungal-infected waxworms over the 72 hour post-exposure period. These results have interesting relevance to our understanding of competition and niche partitioning in entomopathogens. Future investigations will focus on whether these infection decisions are consistent across EPN species with different host ranges and foraging strategies.

N-11 Screening and characterization of *Bacillus thuringiensis* with nematicidal activity against *Ditylenchus destructor*

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D.destructor, which mainly Parasitize in sweet potato, could cause tremendous loss in agricultural production, and the disease caused by this worm has become more and more serious in these years. Traditional control against *D.destructor* includes rotation, application of chemical pesticides and breeding resistant varieties, which has exposed significant limitation because of the low efficiency,high investment and environmental problems. *Bacillus thuringiensis* (Bt), as its specifically high toxicity, harmless to mammals, and safe to environment, has been successfully applicated as microbial insecticides on control of plant parasitic nematodes. But so far, the research on the nematicidal of Bt against D.destructor has rarely been reported. In our study, we tested the nematicidal activities of 12 Bt strains against *D.destructor,* of which YBT-008 showed highest toxicity against *D.*destructor. The four insecticidal genes—cyt2Ba, cry11Aa, cry4Aa and cry4Ba-encoded by YB-008 have been analysed and verified using SDS-PAGE, LC-MS and PCR. Then we cloned their CDS, constructed expression vectors and finally the proteins were expressed in the acrystalliferous Bt strain BMB171. The nematicidal activities of expressed proteins were tested, by which we aimed to acquire new gene and protein resources from Bt strains with high virulence against *D.destructor*. This work was supported financially by the National Basic Research and Development Program (2010CB126600)

N-12 STU Molecular and Biochemical Identification of Symbiotic Bacteria of Entomopathogenic Nematodes Indigenous from Brazil

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Nine species of entomopathogenic nematodes (Nematoda: Rhabditida) isolated from different Brazilian biomes and stored in the collection of the Instituto Biologico, SP, Brazil, were activated in *last-instar* larva of *Galleria mellonella*, for multiplication and symptom observation. Symbiotic bacteria (Enterobacteriaceae) were isolated from the infected hemolymph of *G. mellonella* larvae and from crushed infective juvenile. The bacterial strains were identified phenotypically and biochemically by assessing the absorption of bromothymol blue plus triphenyltetrazolium chloride reduction on NBTA medium; the activities of the enzymes urease, protease and oxidase; the production of acids from glucose, manitol, inositol, sorbitol, rhamnose, sacharose, melibiose, amygdalin and arabinose; the assimilation of glucose, arabinose, mannose, mannitol, N-Acetyl-Glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate, phenylacetic acid; and finally by detectingthe bioluminescence. In addition, we compared partial 16S rDNA sequences from the symbiotic bacteria with sequence from previously described species. The bacteria were characterized biochemically as *Photorhabdus* spp. and *Xenorhabdus* spp. in primary phase, and identified as the following species for each nematode: *P. luminescens (Heterorhabditis indica* CBn5*), P. luminescens (H. amazonensis* AM71*), X. nematophila (Steinernema carpocapse* CBn*2), X. doucetiae (S. brazilense* CBn6*), X. szentirmaii (S. rarum* PAM10*), X. koppenhoeferi* (S.sp CER105), *X.romanii* (*S. puertoricense* CER129), *X. sp*(*S. costaricense* CER17), and *X. sp* (*S. diaprepesi* AM47). The latter two had similarity lower than 96% with other bacteria previously reported in GenBank, which makes necessary the amplification of other genes to confirm whether they belong to new species.

N-13 Entomopathogenic nematodes for the management of black cutworm, *Agrotis ipsilon*, in golf course turfgrass: from lab to optimization in the field

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Seven EPN species were evaluated against different black cutworm instars in the laboratory. In pots with grass, *Steinernema carpocapsae* tended to be the most virulent species followed by *Heterorhabditids bacteriophora*, *H. megidis*, and *S. riobrave*. Fourth and/or fifth instars were the most susceptible stages to most EPN species, pupae the least susceptible. *H. bacteriophora*, *H. megidis*, and *S. carpocapsae* successfully reproduced in fifth and sixth instar and pupa. Field experiments were conducted in golf course fairway-type turf with *H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. riobrave* applied at 1.0 or 2.5×10^9 IJs/ha against fourth instars. *S. carpocapsae* performed the best due to a combination of high control rates and most consistent results (average 83%, range 70–90% at 7 DAT), high speed of kill (average 68% at 4 DAT), and prevention of significant turf damage. *S. feltiae* and *H. bacteriophora* were less consistent and *S. riobrave* was the least effective. To improve EPN consistency, especially under hot conditions, we tested (1) combining two EPN with different host foraging strategies and larval stage preferences, (2) split applications (half rate applied at 0 and 4 DAT vs. full rate at 0 DAT), and (3) syringing (a common practice on golf course greens involving frequent application of low water quantities to reduce heat stress to the grass). Species combinations were not more effective than the better than single species applications. Split applications and syringing tended to improve *S. carpocapsae* and *H. bacteriophora* efficacy. More experiments are being conducted to confirm these trends.

N-14 STU Optimizing the stability and biocontrol efficacy of *Heterorhabditis bacteriophora* using the homozygous inbred line approach

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The consequences of serial culturing are a significant concern in the entomopathogenic nematode (EPN) production industry for both in vivo and in vitro methods. Continuous culturing of biological agents has repeatedly been shown to lead to efficacy reduction due to genetic or non-genetic processes. *Heterorhabditis bacteriophora* is one of the most susceptible species to this problem. The goal of this research project is to use the inbred line procedure as a method to enhance expression of beneficial characters, including cold tolerance and virulence of local EPN strains. The efficacy of homozygous lines (after 7 generations) will be examined in several efficacy trials conducted at various cold temperatures and against several commercial strains.

V-1 STU Developing of a TaqMan quantitative PCR assay for the quantification of *Agrotis* baculoviruses in single and mixed infections

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Four baculoviruses, namely *Agrotis segetum* nucleopolyhedrovirus A (AgseNPV-A), *A. segetum* (Agse) NPV B, *A. ipsilon* (Agip) NPV and *A. segetum* granulovirus (AgseGV) from the genera *Alpha-* and *Betabaculovirus*, respectively, are known to infect larvae of the lepidopteran pests *A. segetum* and *A. ipsilon*. Their potential as biocontrol agents against *Agrotis* species has therefore been examined in dose-response bioassays in order to determine virulence parameters. Under natural conditions, infections often occur as mixed infections between different *Agrotis* baculoviruses, especially between *Agrotis* spp. NPV and AgseGV. In order to obtain a detailed understanding of the mixed infections and the amount of virus progeny produced a quantitative polymerase chain reaction using a SybrGreen-based assay had been developed. However, this method lacks several points, which were improved by the design of highly-specific TaqMan probes for each of the four baculoviruses. The TaqMan probes were designed to bind to the *polyhedrin* gene or *granulin* gene of the *Agrotis* baculoviruses. The TaqMan qPCR assay allows a direct and simultaneous quantification of mixed DNA-species, as each virus is represented by one fluorescing dye. Mixtures of viral DNA samples of AgseNPV-A, AgseNPV-B, AgipNPV and AgseGV have not shown any disturbing influence in the detection of the separate amounts. Hence TaqMan-qPCR-assays might be further used for the examination of viral interactions of baculovirus in experiments with larvae but also tissue cultures.

V-2 Effect of formulation over the efficacy of a *Spodoptera frugiperda* multiple nucleopolyhedrovirus and a genotypic variant.

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The aim of biopesticides formulation is to obtain a final product with stable biological and physical properties, acceptable shelf life, photostability and suitable characteristics for application. In this sense, a Colombian Spodoptera frugiperda MNPV (SfCOL-wt) and a genotypic variant of this virus (SfCOL-A) were formulated by microencapsulating the occlusion bodies with the methacrylic acid polymer Eudragit S100® and an optical brightener. In order to determine the effect of the formulation over viral efficacy, different bioassays were performed with both formulated (SfCOL-A-F, SfCOL-wt-F) and unformulated (SfCOL-A and SfCOL-wt) viruses. Mean lethal concentrations LC_{50} of SfCOL-A (formulated or unformulated) were lower than those obtained for SfCOL-wt, confirming the higher pathogenicity of SfCOL-A; however formulation did not affect pathogenicity of both viruses under laboratory conditions. Then, formulated and unformulated viruses were irradiated for six hours with monochromatic UV-B, treatment which caused an inactivation of 95% to SfCOL-A and 55% to SfCOL-wt, while formulated viruses were not inactivated, maintaining efficacies higher than 90%, which could increase the persistence of virus in field conditions. Efficacies higher than 80% were obtained with chemical insecticide lufenuron, SfCOL-A, SfCOL-A-F and SfCOL-wt-F under greenhouse conditions and differences were not found between insecticidal activities of formulated and unformulated SfCOL-A. However, SfCOL-wt-F showed a significantly higher efficacy than unformulated virus, confirming that developed formulation improves viral performance.

V-3 A survey of honey bee viruses in Iowa reveals variation in virus species prevalence across apiaries, bee development, and season

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Honey bee virus infections have been suspected as a potential cause of colony collapse disorder (CCD), a syndrome characterized by the sudden disappearance of adult honey bees. However, it is becoming widely accepted that CCD involves multiple factors. The present work is framed around studying how the interaction of viral infections and nutrition affects honey bee health. To begin investigating these factors, we performed a multi-year screening of Iowa apiaries for several honey bee viruses including Israeli acute paralysis virus (IAPV), Deformed wing virus (DWV) and Black queen cell virus (BQCV), among others. DWV and BQCV were the predominant viruses found in virtually all samples, independent of year and colony health. To further characterize viral infections across and within hives, we also screened hives from a research apiary in order to compare several developmental stages and time points. Our preliminary results showed adult bees test positive for more viral species than juvenile stages. Viral titer and diversity varied within each colony between sampling time points. Additionally, higher viral diversity and titer were observed in weak colonies. These results reflect a complex dynamic network of virus strains and virulence thresholds which are currently under investigation.

V-4 Discovery and complete genome sequence of a new iflavirus covertly infecting a cell line derived from the gypsy moth (*Lymantria dispar*, Linnaeus).

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Cell cultures derived from the gypsy moth are routinely used to study insect virus-host interactions. We attempted to use the cell line IPLB-Ld652Y for study of a small RNA virus in the *Dicistroviridae*. Interestingly, we found another virus of similar shape and size to the dicistrovirus under investigation. The new virus, which we call Lymantria dispar virus 1 (LyDV1), has icosahedral virions of approximately 30 nm in diameter. LyDV1 increases in abundance when cells are stressed. The 10 kb polyadenylated RNA genome of LyDV1 has a predicted 0.9 kb 5' UTR, an ORF of 8.9 kb and a 3' UTR of 0.2 kb. The predicted polyprotein of nearly 3,000 amino acids shares highest identity (36 and 37%) with the polyproteins of Varroa destructor virus and Deformed wing virus in the *Iflaviridae* in the *Picornavirales*. These results provide a warning for virologists using cell lines that may contain covert viral infections. Such viruses may alter cellular conditions or express trans-acting viral proteins that affect replication of challenging viruses leading to misinterpretation of results. Our work also further demonstrates the abundance and ubiquity of picorna-like viruses.

V-5 Isolation and genetic analysis of a cypovirus from the bilobed looper, *Autographa biloba*

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Cypoviruses are non-enveloped arthropod-specific viruses of the family Reoviridae with a segmented, double-stranded RNA genome. These viruses complete their replication cycle exclusively in the cellular cytoplasm by extruding mRNA and daughter genomes from conserved "turret" structures in their capsids. Field-isolated bilobed loopers (*Autographa biloba*) from Rolla, Missouri, that had succumbed to lethal baculovirus infection with *Anagrapha falcifera* multicapsid nucleopolyhedrovirus (AfMNPV) were found to also be co-infected with a cypovirus. Occlusion bodies containing both baculovirus and cypovirus particles were recovered from experimentally infected larvae. Liberation of the associated nucleic acids revealed a high molecular weight DNA band corresponding to the baculovirus genome and a ten-segmented dsRNA cypovirus genome. Further analysis revealed that the ten segments consisted of 0.5 to 4.5k base-pair bands, comprising a total genome size of approximately 22kb. Interestingly, co-infection of *Heliothis virescens* larvae with a mixture of the baculovirus and cypovirus did not result in extensive differences in mortality compared to infection with analogous preparations containing baculovirus alone. Partial sequencing of DNA constructed from the cypovirus dsRNA genome indicates that this is a type 5 cypovirus bearing remarkable homology to that of *Heliothis armigera* cypovirus (HaCPV), previously isolated in China. Complete genomic sequencing of this cypovirus and further studies on its interactions with baculoviruses will yield new insights into the pathogen-host interplay in the field and possibly new tools for use in crop protection strategies.

V-6 Functional Analysis and Improvement of cellulases produced by recombinant baculovirus

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Cellulosic materials are the most abundant renewable bioenergy resources on earth waiting for exploration. In nature, wood feeding insects can secret a combination of cellulases for efficient digestion of the cellulosic materials. Although a high number of endoglucanases have been found in these xylophagous insects, little is known about exoglucanases encoded in the genome of these insects. Here we report the identification and isolation of an exoglucanase, designated as AmCel-5B, from the white spotted longhorn beetle, *Anoplophora malasiaca*. Interestingly, this enzyme is not only exhibit exo-β-glucanase activity, but also with obvious endo-β-glucanase activity. Uniquely, although it recognizes Avicel, evidenced as an exo-β-glucanase, it cannot recognize oligosaccharides smaller than cellohexaose. This may explain why longhorn beetle can well digest hard "living" wood, which contains primarily rigid long fibers. Furthermore, we also improved the stability and activity of a thermophilic GH5 endo-glucanase GsCelA by structure-guided recombination. In these experiments, the sequence-function relationships were first analyzed and predicted by a set of eight trial synthetic GH5 enzymes. Four synthetic enzymes were further synthesized to test these predictions. Among them, a chimera with a significant increase in thermostability for 4 °C, and in activity for 20%, comparing with native GsCelA, was found. Furthermore, five mutated amino-acid residues of this chimera and a novel stabilizing loop are identified by site-directed mutagenesis. Our cellulase study facilitates potential applications of these novel enzymes in the production of bioenergy and biomaterials from lignocellulosic biomass in the future.

V-7 Sequence of a novel iflavirus identified in the transcriptome of the brown marmorated stink bug, *Halyomorpha halys*

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The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Hemiptera: Pentatomidae), is an invasive species from Asia that has become a severe agricultural pest in the mid-Atlantic US, where it attacks a wide range of fruits, vegetables, and other host plants and also acts as a nuisance pest that infests homes in large numbers during the fall and winter. Determination of the BMSB transcriptome

revealed sequencing reads sharing >70% nucleotide sequence identity with a picorna-like virus detected in fecal samples from the big brown bat, *Eptesicus fuscus*. These reads assembled into a 9,271 nt transcript terminating in a poly(A) tail and containing a large 9,051 nt ORF. Phylogenetic inference with an RNA-dependent RNA polymerase (RdRp) domain encoded by the ORF and with conserved iflavirus and dicistrovirus RdRp domains grouped the BMSB sequence with other iflavirus sequences, with the closest relationships observed with the *E. fuscus* fecal sample picorna-like virus and with sacbrood virus. The presence of the iflavirus genome in BMSB RNA samples was confirmed by PCR with first-strand cDNA primed with either oligo-dT or gene-specific primers. 5'- and 3'-terminal sequences were confirmed by 5' and 3' RACE. The proportion of total transcriptome reads corresponding to the BMSB iflavirus sequence increased by approximately 480-fold in BMSB adults relative to BMSB nymphs, suggesting increased replication of the viral genome occurring sometime after the nymph-adult molt. This research points to a pathogen that potentially can be exploited for control of BMSB populations.

V-8 Identification and Characterization of protease activity of nonstructural protein 3C and RNA chaperone activity of 2C

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Picorna-like viruses in the *Picornavirales* order are a large group of positive-strand RNA viruses that include numerous important pathogens for plants, insects, and humans. Viral replication and capsid assembly in the viruses in the order Picornavirales requires polyprotein proteolytic processing by 3C or 3C-like (3CL) proteases. We identified and characterized the 3CL protease of Ectropis obliqua virus (EoV) of the newly established family Iflaviridae (order Picornavirales). The bacterially expressed EoV 3CL protease domain autocatalytically released itself from larger precursors by proteolytic cleavage, and cleavage sites were determined via N-terminal sequencing of the cleavage products. This protease also mediated trans-proteolytic activity and cleaved the polyprotein at the same specific positions. EoV protein 2C is one of the most conserved proteins, which contains an ATPase activity and a putative RNA helicase activity. We determined that 2C contains the RNA chaperone activity. Our further characterization of EoV 2C revealed that divalent metal ions, such as Mg^{2+} and Zn^{2+}, inhibit 2C-mediated helix destabilization with different extents. Moreover, we determined that EoV 2C also contains ATPase activity like other picornaviral 2C proteins, and further assessed the functional relevance between its RNA chaperone-like and ATPase activities using mutational analysis as well as their responses to Mg^{2+}. Our work is the first study to identify an iflaviral 3CL protease and further characterize it in detail. Also, the characterization of 2C RNA chaperone activity may be critical for picornaviral replication and pathogenesis, and should foster our understanding of picorna-like viruses and viral RNA chaperones.

V-9 A cell line derived from the glassy-winged sharpshooter, *Homalodisca vitripennis*, supports replication of viral RNA from a clone of *Homalodisca coagulata* virus 1.

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Pierce's disease is a devastating disease of grapevines caused by the bacterium *Xylella fastidiosa* that is transmitted to plants by the glassy winged sharpshooter (GWSS), *Homalodisca vitripennis* (Hemiptera: Cicadellidae). Methods are under investigation for modifying or enhancing the insecticidal activity of a naturally occurring dicistrovirus (*Homalodisca coagulata* virus-1 or HoCV-1) infective to GWSS. *In vitro* transcribed RNA from three plasmid clones carrying wild-type (pT7-HoCV1-3'Rz, pT7-Rz-HoCV1-3'Rz) or mutated (pT7-Rz-mutHoCV1-3'Rz) HoCV-1 viral sequences was transfected into GWSS-Z15 cells derived from *H. vitripennis*. RNA generated from the pT7-HoCV1-3'Rz construct caused cytopathic effects and replicated in GWSS-Z15 cells whereas RNA generated from other clones was inactive. Transcripts generated with or without a 5' CAP on the sequence were fully infectious for the pT7-HoCV1-3'Rz construct with an up to 200 fold increase in positive and negative sense HoCV-1 viral RNA transcript quantities detected by day 2 post-transfection. Relative levels of negative sense RNA were approximately 10 fold lower than positive sense RNA at their peak. We are currently examining HoCV1-3'Rz infected GWSS cells by electron microscopy and investigating the infectivity of clone-generated HoCV-1 particles in *H. vitripennis*. Future experiments will target recombinant RNAi-expressing HoCV-1 particles for enhanced infectivity and lethality in the leafhopper host.

V-10 Baculovirus prevalence in western spruce budworm (*Choristoneura occidentalis*) populations in British Columbia, Canada

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Population studies of western spruce budworm have been conducted over a broad geographical area of British Columbia for more than a decade. These data reveal that an alphabaculovirus (ChocNPV) is widespread in outbreak populations although the rate of mortality is usually very low (<5%). In 2006, rates of mortality from ChocNPV increased and exceeded 40% in some sites. Rates also varied with forest condition with more mortality in dense, as compared to open, stands. Since 2006, rates of virus-caused mortality have decreased, as has overall density of budworm larvae in long-term study sites. Elevated levels of virus mortality, however, can be regularly found wherever populations reach very high densities (approx. 300 insects per kg of foliage) and appear to contribute to rapid rates of decline in these areas. In 2010, subsamples of live budworms were examined with molecular probes to estimate the relative frequency of infected budworms among the live population at the time of sampling. Special attention was paid to new, high-density populations. Rates of infection estimated with this method were closely correlated to those obtained by only diagnosing insects that had died. These new results suggest that while virus is present in most populations, a significant increase in frequency depends strongly on horizontal infection rates that are, in turn, associated only with very high densities of feeding budworms.

V-11 Baculoviridae: toward understanding the mechanism of replication

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Baculoviruses are arthropod-specific viruses containing large double-stranded circular DNA genomes of 80,000–180,000 bp. The progeny generation is biphasic, with two different phenotypes during virus infection. These viruses have been used in many biological

applications. Until day, is not completely known if baculoviruses has one or more origins, or which are the molecular pathways associated (theta, rolling circle, recombination processes). In this work, with the goal to add more experimental evidence that allow understand the baculovirus replication, a bioinformatic and experimental approach were applied using AgMNPV and UFL-Ag-286 cells as model. Thus, the structure of replication intermediates of virus genomes was studied by partial restriction enzyme digestion of intracellular replicated DNA resolved by FIGE and hybridization. On the other hand, to evaluate if homologous recombination is involved in concatemer resolution a transient transfection assay was done using bacterial plasmids and infections processes. Besides, the identification of origin sequences was carried out by other transient replication assay based on the transfection of virus DNA fragments cloned in bacterial plasmids together with infection processes. To obtain those plasmids, AgMNPV genome were segmented in 2 ways: first, in 9 big fragments, or second by partial digestion with the enzyme HaeIII. The levels of plasmid multiplication in infected cells were estimated by quantitative Real Time-PCR and compared among them. All the results were discussed and confronted with proper bioinformatic studies with the goal to postulate putative mechanisms involved in Baculoviridae replication.

V-12 The genome of an entomopoxvirus of the genus *Alphaentomopoxvirus*, including its terminal hairpin loop sequences, with a possible unique apoptosis inhibition system

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Entomopoxviruses (EVs) are divided into three genera, *Alphaentomopoxvirus*, *Betaentomopoxvirus* and *Gammaentomopoxvirus*. We for the first time sequenced the complete genome of an *alphaentomopoxvirus* virus. The genome of this virus, *Anomala cuprea* EV (ACEV), shown here includes its both terminal hairpin loop structures, which is the first description of EV hairpin loop structures. The genome excluding its hairpin loops is 245, 717 bp in length, which is smaller than the genome size of *Alphaentomopoxvirus* previously considered, and consists of a central coding region bounded by inverted terminal repeats (ITRs) of 22,978 bp that is the longest ITRs among wild type of poxviruses previously reported. The hairpin loop at each end of two DNA strands showed two types of sequences that consist of inverted repeat sequences between the two. The genome contains 286 open reading frames, and some, including a translation initiation factor eLF-4E and phosphatidic acid phosphatase type 2, may be transferred from eucaryotes because of their lack in known poxvirus genomes. We found an ORF which contains a serine protease inhibitor (serpin) domain sequence that has been found in vertebrate poxviruses but not in EVs. Furthermore, two different domains (BIR, RING) in an ORF of inhibitor of apoptosis (IAP), that was found in insect viruses (baculoviruses and other EVs) are separated in two different ORFs that are distant from each other on the genome, suggesting that ACEV harbors different apoptosis-inhibition system from that in known EVs.

V-13 Genetic variation and biological activity of isolates of *Lymantria dispar multiple nucleopolyhedrovirus* from North America, Europe, and Asia

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Little is known about genetic variation of *Lymantria dispar multiple nucleopolyhedrovirus* (LdMNPV; *Baculoviridae*: *Alphabaculovirus*) at the nucleotide sequence level. To obtain a more comprehensive view of genetic diversity among isolates of LdMNPV, partial sequences of the *lef-8* gene were generated by PCR of 18 *L. dispar* virus samples isolated from the geographic range of the gypsy moth. Phylogenetic inference revealed a group of Asian LdMNPV isolates that formed a clade separate from LdMNPV isolates from Europe and North America. The complete genome sequence was determined for an isolate from this group, LdMNPV-2161 (Korea). The LdMNPV-2161 genome was 163,138 bp in length, 2,092 bp larger than the genome of LdMNPV isolate Cl5-6 (CT, USA). The two genomes shared an overall nucleotide sequence identity of 97.5% with 566 gaps inserted for alignment optimization. The difference in genome size was due primarily to additional members of the *baculovirus repeated orf* (*bro*) gene family in LdMNPV-2161 and the presence of a previously reported deletion of the *p24* ORF and an adjacent ORF in LdMNPV Cl5-6. In bioassays against the New Jersey Standard Strain of *L. dispar*, isolates LdMNPV-3029 (Russia) and LdMNPV-Ab-a624 (MA, USA) killed neonate larvae with an LC_{50} approximately 2.5-fold lower than a sample of Gypchek® and isolates LdMNPV-3041 (Japan) and LdMNPV-2161 (Korea). This study expands our knowledge about about genetic variation among LdMNPV isolates and provides novel information on the distinct groups in which these NPVs occur.

V-14 Ultrastructure analysis of six insect cell lines infected with *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV)

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The *in vivo* production of *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) has been limited by cannibalism and also by the strong cuticle lyses of the infected larva. In order to develop studies on baculovirus *in vitro* production, six insect cell lines were tested regarding its susceptibility to this virus. Initially, cells were incubated with SfMNPV I-19 isolate (budded virus) for 1h adsorption time, and kept in TNMFH medium with 10% FBS at 27^0C. The cytopathic effects were monitored daily by light microscopy. Infected and mock infected cells were then pelleted at 4 d.p.i. and processed for transmission electron microscopy. Morphological analysis by light microscopy showed that infected IPLB-SF-21AE and Sf9 cells lead to successful viral replication with many polyhedra formation. However, *Lymantria dispar* cells (IPLB-LD-625Y) became highly vacuolated while *Bombyx mori* cells (BM-5) seemed to change its morphology from round refractive to "groundnut-shape", although none of them showed polyhedra production. On the other hand, there were no morphological changes in *Anticarsia gemmatalis* (UFL-AG-286) and in *Trichoplusia ni* (BTI-Tn-5B1-4) cells. As expected, ultrastructural analysis of the two *Spodoptera frugiperda* infected cell lines revealed typical baculovirus induced effects such as cell nucleus hypertrophy and the presence of virogenic stroma, nucleocapsids, virions and occlusion bodies. None of the other cell lines showed virus particles inside the cell nucleus. Nevertheless, *Lymantria dispar* cells showed several vesicles and vacuoles in the cytoplasm. In conclusion, only *S. frugiperda* cells (IPLB-SF-21AE and Sf9) were susceptible to SfMNPV and are suitable candidates for its *in vitro* production.

V-15 Accumulation of Wuhan Nodavirus genomic RNA template requires membrane association of protein A

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One common feature of positive-strand RNA viruses is the association of viral RNA and viral RNA replicase proteins with specific intracellular membranes to form RNA replication complexes. Wuhan nodavirus (WhNV) encodes protein A, which is the sole viral RNA replicase. Here, we showed that WhNV protein A closely associates with mitochondrial outer membranes and colocalizes with viral RNA replication sites. We further identified the transmembrane domains (N-terminal aa 33-64 and aa 212-254) of protein A for membrane association and mitochondrial localization. Further investigation show that aa 34-64 and aa 212-254 could function as independent mitochondrial localization signals and further elucidated that the aa 33-64 and aa 212-254 regions are sufficient for WhNV protein A membrane association and mitochondrial localization. Moreover, we found that WhNV protein A efficiently stabilizes viral genomic RNA independent of its RNA replicase activity. And our further investigation revealed that WhNV protein A accumulates viral genomic RNAs by recruiting them to intracellular membrane sites, and this process is closely coupled to the membrane association of protein A. This study represents an advance toward understanding the mechanism of the RNA replication of WhNV and probably other nodaviruses.

V-16 STU Novel ascovirus isolated from *Spodoptera litura* in Japan

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Ascoviruses (AVs) are double-stranded DNA viruses mainly infect lepidopteran larvae in the family Noctuidae. AV isolates have been reported from U.S, some Europe and Asian countries and Australia, but not from Japan. In this study, we collected the oriental leafworm moth, *Spodoptera litura* (Lepidoptera: Noctuidae), larvae from organic farms in an attempt to isolate AVs. Among 811 larvae collected in Saitama Prefecture, two larvae showed typical symptom of AV as stunted growth and white-colored hemolymph with virion-containing-vesicles. The isolate was designated as *S. litura* AV (SlAV). Pathogenicity was examined either orally or intra-hemocoelically with virion suspension. Since SlAV was highly infectious intra-hemocoelically, it had no *per os* infectivity. Sequence analysis of SlAV for major capsid protein, metalloprotease and ATPase revealed that SlAV was highly similar to *Heliothis virescens* AV 3e (HvAV-3e), but the sequence was not identaical. Other than *S. litura*, SlAV was infectious to larvae of *Mythimna separata* (Lepidoptera: Noctuidae) and *Spodoptera exigua*. AVs are generally transmitted by ovipositor of endoparasitic wasps. In the field in Saitama, endoparasitoid *Meteorus pulchricornis* (Hymenoptera: Braconidae) was prevalent and female of this species may play a role as vector of SlAV. According to laboratory study for exposure of the female *M. pulchricornis* to SlAV-infected *S. litura* larvae, SlAV was transmitted by *M. pulchricornis*.

V-17 STU How does AcMNPV virulence change following experimental evolution in different hosts?

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The occlusion bodies (OBs) of multiple nucleopolyhedrovirus (MNPVs) contain many virions, each enclosing several nucleocapsides. OBs therefore contain populations of genomes. This structure allows MNPVs to maintain high genetic variation, on which natural selection acts for adaptation to particular host species. The signature of adaptive evolution is the genetic and phenotypic differentiation of viral populations, observable through genomic and virulence changes. We conducted experimental evolution on a polymorphic wild type AcMNPV population by ten serial passages on the semi-permissive host *Spodoptera exigua* in 10 replicates. These 10 viral lines are expected to have adapted to *S exigua*. To assess their phenotypic evolution, we challenged the 10 lines on *S. exigua* and *Trichoplusia ni*. We infected 10 caterpillars with 3 virus doses (50, 5 000 and 500 000 OBs). Deaths were followed for 22 days post-infection. Virulence was analyzed in terms of lethal time, lethal dose and yield. We found different evolutionary trajectories had been selected for the adaptation of the 10 AcMNPV lines to *S. exigua*. Challenge on *S.exigua* showed 9 out of 10 lines were more virulent than the wild-type virus, while 1 line was less virulent. Challenge on *T.ni* showed 6 lines were close to the wild-type virus while 4 strains had increased in virulence. Here, we found that controlled ecological adaptation to a particular host leads to the evolution various phenotypes. The majority of viral lines converged towards the same virulence phenotypes, but extraordinary phenotypes also emerged.

V-18 STU Characterization of the role of baculovirus sulfhydryl oxidases in virion morphogenesis

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The *Autographa californica* M nucleopolyhedrovirus (AcMNPV) *ac92* gene encodes a flavin adenine dinucleotide-linked sulfhydryl oxidase related to the ERV/ALR family of sulfhydryl oxidases. A mutant with substitutions in the predicted Ac92 sequence $C^{155}XXC^{158}$, important for sulfhydryl oxidase activity in cellular enzymes, is unable to oxidize substrates. Previously, we showed that recombinant AcMNPV bacmids with a deletion in *ac92* or substitutions in the $C^{155}XXC^{158}$ sequence did not produce infectious budded virus (BV). We were able to detect viral structural proteins and viral DNA in the supernatants of cells transfected with *ac92*-deleted bacmid DNA, indicating that BV is produced in the absence of *ac92*, but these BV have defects in infectivity. We are currently exploring the role of *ac92* in the production of infectious BV. In addition, AcMNPV bacmids with mutations in *ac92* produced singly-enveloped occlusion derived virions (ODV) instead of multiply-enveloped ODV. To gain insight into how *ac92* affects the formation of multiply-enveloped ODV, we inserted the *ac92* ortholog from *Trichoplusia ni* SNPV, *tn79*, a virus that produces singly-enveloped ODV, into an AcMNPV bacmid lacking *ac92*. We are determining if *tn79* is able to substitute for *ac92* and rescue the defects of a virus lacking *ac92* in BV infectivity and ODV morphology.

V-19 STU Next Generation Sequencing to Identify Genetic Diversity within Baculovirus Isolates

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An SNPV from *Helicoverpa* spp. originating in Brookstead, Queensland, Australia is widely used as a commercial biopesticide in Australia and South Africa, and has been used as a model for *in vitro* production studies. Two samples of the virus were obtained: the first was a single passage from the original isolate while the second from the University of Queensland (UQ) had been passaged in *H. armigera* larvae. Both isolates were passaged once in *H. armigera* larvae that had been shown by PCR to be free of potential contaminating cryptic infection. Denaturing gel electrophoresis (DGGE) of genes *me53*, *dbp1* and *DNA polymerase* was used to detect viral genotypes in each sample. Ion Torrent PGM™ semiconductor sequencing was used to generate *de novo* sequence of the virus genome and to identify regions of genetic diversity in the population of strains within each

isolate. Sequencing identified the Brookstead isolate as more similar to the *H. zea* SNPV reference genome (NC_003349) than to the 4 *Hear*SNPV genomes NNg1, G4, C1 and an unnamed Australian isolate (JN584482). This supports the classification of Heliothine SNPVs as a single species. Next generation sequences were used to identify hypervariable regions, deletions and insertions. The relative utility of DGGE and next generation sequencing in detecting strain variation within baculovirus isolates is discussed.

V-20 STU Phylogenetic relationship between pale tussock moth nucleopolyhedrovirus (DuPuNPV) and other Lymantriidae baculoviruses.

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The pale tussock moth (*Dasychira pudibunda*) belonging to the family of *Lymantriidae*, is a pest of deciduous trees (oak, beech, hazel) that occurs all over Europe. Caterpillars of this moth attack healthy trees which make them the primary pest. During late summer and autumn large occurrence of the pale tussock can lead to mass defoliations and serious weakening of trees. One of the main natural enemies of the moth is the baculovirus DapuNPV. Baculoviruses-based biopesticides are regarded as very safe for human and animals, because they infect only arthropods and do not replicate outside their natural host. To get better insight into DapuNPV molecular biology and its genetic correlation with other baculoviruses we determined and analyzed eight full length genes (chitinase, DNA polymerase, polyhedrin, gp64, IE-1, LEF-9, LEF-8). We found them very similar to those of the Douglas-fir tussock moth (*Orgyia pseudotsugata*) and the white satin moth (*Leucoma salicis*) baculoviruses which have a big potential for pest occurrence controlling. In this presentation, after detailed DNA sequencing and phylogenetic analysis, we show genetic relationships between DupuNPV and many other baculoviruses.

V-21 STU *In vitro* and *in vivo* tranfection to construct recombinant *Adoxophyes honmai* nucleopolyhedrovirus particles

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The smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae), is susceptible to two nucleopolyhedroviruses (NPVs), *A. honmai* NPV (AdhoNPV) and *A. orana* NPV (AdorNPV), which are genetically closely related but differ in killing speed. To elucidate genes related to killing speed of *Adoxophyes* NPVs, construction of an AdhoNPV bacmid system was needed and a recombiant AdhoNPV bacmid DNA, expressing enhanced green fluorescent protein (EGFP) gene under the heat shock promoter (AdhoNPV-hsp-EGFP), was constructed. In general, bacmid DNA is transfected to permissive cell line and infectious virions can be obtained, however, AdhoNPV has no permissive cell line. To obtain recombinant AdhoNPV particle, condition of *in vitro* and *in vivo* transfection, using cell lines and host insects, was examined. Efficacy of transfection was detected by expression of a gene marker, *egfp*, and virus genes, AdhoNPV *ie-1* and *polh*. Green fluorescence and viral gene expressions were detected from *in vitro* transfected non-permissive cell lines, AhL83 derivered from *A. honmai* and Sf9. On the other hand, for *in vivo* transfection, because *A. honmai* larvae were too small to be injected hemocoelically, methoprene (juvenile hormone analog: JHA) was applied to enlarge the larval size and extend the larval period. However, no viral expression was detected from the bacmid-injected larvae using similar condition with *in vitro* transfection.

V-22 STU Cross-resistance to a granulovirus, an entomopoxvirus and *Bacillus thuringiensis* of the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae) selected for resistance to the nucleopolyhedorvirus of *A. honmai*

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The smaller tea tortrix, *Adoxophyes honmai*, is the important pest of tea cultivation in Japan. *A. honmai* has acquired resistance against chemical pesticides, and biological control is now used to control it. To determine whether *A. honmai* can also acquire resistance against baculovirus agents, a field-collected *A. honmai* population was selected with a 70% lethal concentration (LC_{70}) of *A. honmai* nucleopolyhedrovirus (AdhoNPV) in the laboratory. The selection was carried out for 16 years, and after 155 generations AdhoNPV-selected strain (resistant strain; R-strain) showed over 10,000-fold higher resistance against AdhoNPV than the nonselected strain (susceptible strain; S-strain). We examined the cross-resistance of the R-strain against *Adoxophyes orana* granulovirus (AdorGV), *A. honmai* entomopoxvirus (AHEV) and *Bacillus thuringiensis*. Neonate larvae of the R-strain and the S-strain were exposed to AHEV and AdorGV by the droplet feeding method and the LC_{50} values for AHEV and AdorGV were compared between the two strains by probit analysis. The LC_{50} value of the R-strain for AHEV was significantly three times higher than that for the S-strain. For AdorGV the LC_{50} value of the S-strain for the R-strain were significantly 30 - 182 times higher than those for the S-strain. These results suggested that the R-strain showed cross-resistance to AHEV and AdorGV. When the 3rd instar larvae of the R-strain and the S-strain were exposed to XenTari® (Sumitomo Chemical Co. Ltd.), the biological insecticide based on *B. thuringiensis* serovar *aizawai*, LC_{50} of the two strains did not differ significantly based on comparison of 95% confidence limits of LC_{50}.

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Hall, Spencer	4	Plenary	Mon., 12 Aug	12:00
Han, Ji Hee	F-13	Poster Session	Wed., 14 Aug	10:30 - 15:30
Harrison, Robert L.	161	Viruses 4	Thurs., 15 Aug	8:00
Harrison, Robert L.	V-7	Poster Session	Wed., 14 Aug	10:30 - 15:30
Hayakawa, Tohru	63	Bacteria 2	Tues., 13 Aug	8:00
Hazir, Selcuk	72	Nematodes 2	Tues., 13 Aug	8:30
Hazir, Selcuk	N-3	Poster Session	Wed., 14 Aug	10:30 - 15:30
He, Kanglai	B-26	Poster Session	Wed., 14 Aug	10:30 - 15:30
Heckel, David G.	159	Bacteria Symposium	Thurs., 15 Aug	9:00
Heinig, Rebecca	135 STU	Microbial Control 2	Wed., 14 Aug	16:00
Henderson, Deborah E.	38	Microbial Control 1	Mon., 12 Aug	16:45
Herniou, Elisabeth A	109	Viruses 2	Wed., 14 Aug	9:15
Hertlein, Gillian	87 STU	Diseases of Beneficial Invertebrates 1	Tues., 13 Aug	10:45
Hesketh, Helen	180	Cross Divisional Symposium	Thurs., 15 Aug	14:00
Howlader, Mohammad T. H.	150	Bacteria 3	Wed., 14 Aug	16:15
Hu, Xiaomin	151	Bacteria 3	Wed., 14 Aug	16:30
Hu, Yan	152	Bacteria 3	Wed., 14 Aug	16:45
Hu, Yuanyang	V-8	Poster Session	Wed., 14 Aug	10:30 - 15:30
Huang, Huachao	164 STU	Viruses 4	Thurs., 15 Aug	8:45
Huang, Wei-Fone	128	Microsporidia 1	Wed., 14 Aug	14:00

Humber, Richard A.	92	Fungi & Microbial Control Workshop	Tues., 13 Aug	11:30
Humber, Richard A.	95	Fungi Symposium	Wed., 14 Aug	8:30
Ishii, Minehiro	F-32 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Jackson, Jerreme	B-24 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Jackson, Trevor	189	Microbial Control 3	Thurs., 15 Aug	15:00
James, Rosalind R.	88	Diseases of Beneficial Invertebrates 1	Tues., 13 Aug	11:00
Jaronski, Stefan T.	115	Cross Divisional Symposium	Wed., 14 Aug	14:00
Jaronski, Stefan T.	F-14	Poster Session	Wed., 14 Aug	10:30 - 15:30
Jehle, Johannes A.	116	Cross Divisional Symposium	Wed., 14 Aug	14:30
Johny, Shajahan	59	Fungi 3	Tues., 13 Aug	9:00
Johny, Shajahan	F-15	Poster Session	Wed., 14 Aug	10:30 - 15:30
Jurat-Fuentes, Juan Luis	22	Bacteria 1	Mon., 12 Aug	15:15
Kanost, Michael R.	5	Cross Divisional Symposium	Mon., 11 Aug	14:00
Kaplan, Fatma	N-2	Poster Session	Wed., 14 Aug	10:30 - 15:30
Kepler, Ryan M.	93	Fungi & Microbial Control Workshop	Tues., 13 Aug	12:00
Kepler, Ryan M.	F-16	Poster Session	Wed., 14 Aug	10:30 - 15:30
Keyser, Chad A.	F-33 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Keyser, Chad A.	MC-15	Poster Session	Wed., 14 Aug	10:30 - 15:30
Khajuria, Chitvan	39	Microbial Control 1	Mon., 12 Aug	17:00
Kim, Jae Su	46	Fungi 2	Mon., 12 Aug	17:15
Kim, Jeong Jun	F-17	Poster Session	Wed., 14 Aug	10:30 - 15:30
Kleespies, Regina G.	193	Viruses 5	Thurs., 15 Aug	14:45
Klingen, Ingeborg	60	Fungi 3	Tues., 13 Aug	9:15
Klinger, Ellen G.	89 STU	Diseases of Beneficial Invertebrates 1	Tues., 13 Aug	11:15
Koike, Masanori	MC-7	Poster Session	Wed., 14 Aug	10:30 - 15:30
Koike, Masanori	MC-8	Poster Session	Wed., 14 Aug	10:30 - 15:30
Koppenhöfer, Albrecht M.	10	Nematodes 1	Mon., 11 Aug	14:15
Koppenhöfer, Albrecht M.	N-13	Poster Session	Wed., 14 Aug	10:30 - 15:30
Krejmer, Martyna	82 STU	Viruses 1	Tues., 13 Aug	11:30
Kuhar, Daniel	B-10	Poster Session	Wed., 14 Aug	10:30 - 15:30
Kyei-Poku, George	125	Microsporidia 1	Wed., 14 Aug	13:30
Kyei-Poku, George	M-2	Poster Session	Wed., 14 Aug	10:30 - 15:30
Lazzaro, Brian P.	1	Plenary	Mon., 12 Aug	10:30
Lee, Se Jin	43 STU	Fungi 2	Mon., 12 Aug	16:30
Leite, Luis G.	N-4	Poster Session	Wed., 14 Aug	10:30 - 15:30
Leland, Jarrod	MC-9	Poster Session	Wed., 14 Aug	10:30 - 15:30
Lemes, Ana Rita N.	B-25 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Lemos, Manoel Victor F.	B-11	Poster Session	Wed., 14 Aug	10:30 - 15:30
Li, Haitao	64	Bacteria 2	Tues., 13 Aug	8:15
Liao, Xinggang	F-18	Poster Session	Wed., 14 Aug	10:30 - 15:30
Link, Carolyn	54	Diseases of Beneficial Invertebrates Symposium	Tues., 13 Aug	9:20
Lira, Justin	B-12	Poster Session	Wed., 14 Aug	10:30 - 15:30
Liu, Sijun	156	Viruses Workshop	Wed., 14 Aug	21:00
Liu, Yang	165 STU	Viruses 4	Thurs., 15 Aug	9:00

Loker, Eric (Sam)	6	Cross Divisional Symposium	Mon., 11 Aug	14:30
Lovett, Brian R.	139 STU	Microbial Control 2	Wed., 14 Aug	17:00
Loy, Duan	168 STU	Viruses 4	Thurs., 15 Aug	9:45
Lu, Hsiao-ling	47	Fungi 2	Mon., 12 Aug	17:30
Lucarotti, Christopher	V-10	Poster Session	Wed., 14 Aug	10:30 - 15:30
Luck, Ashley N.	N-5	Poster Session	Wed., 14 Aug	10:30 - 15:30
Luke, Belinda	140	Microbial Control 2	Wed., 14 Aug	17:15
Luke, Belinda	188	Microbial Control 3	Thurs., 15 Aug	14:45
Luo, Zhibing	170	Fungi 4	Thurs., 15 Aug	8:15
Maharramov,Jafar	121	Diseases of Beneficial Invertebrates 2	Wed., 14 Aug	14:15
Maniania, Nguya K.	27	Fungi 1	Mon., 12 Aug	14:30
Manns, Shawn	MC-10	Poster Session	Wed., 14 Aug	10:30 - 15:30
Maxwell, Danica F.	179 STU	Nematodes 3	Thurs., 15 Aug	9:15
Mc Namara, Louise	F-34 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
McMullen II, John G.	N-6	Poster Session	Wed., 14 Aug	10:30 - 15:30
Meeus, Ivan	122	Diseases of Beneficial Invertebrates 2	Wed., 14 Aug	14:30
Meeus, Ivan	DB-1	Poster Session	Wed., 14 Aug	10:30 - 15:30
Meyling, Nicolai V.	F-19	Poster Session	Wed., 14 Aug	10:30 - 15:30
Miele, Solange Ana Belen	V-11	Poster Session	Wed., 14 Aug	10:30 - 15:30
Mitsuhashi,Wataru	V-12	Poster Session	Wed., 14 Aug	10:30 - 15:30
Moar, William	153	Bacteria 3	Wed., 14 Aug	17:00
Molloy, Daniel	53	Diseases of Beneficial Invertebrates Symposium	Tues., 13 Aug	9:00
Molloy, Daniel P.	118	Diseases of Beneficial Invertebrates 2	Wed., 14 Aug	13:30
Molloy, Daniel P.	124	Diseases of Beneficial Invertebrates 2	Wed., 14 Aug	15:00
Monserrate, Jessica P.	B-13	Poster Session	Wed., 14 Aug	10:30 - 15:30
Montalva, Cristian	61 STU	Fungi 3	Tues., 13 Aug	9:30
Morris, E. Erin	11	Nematodes 1	Mon., 11 Aug	14:30
Morris, E. Erin	177 STU	Nematodes 3	Thurs., 15 Aug	8:45
Murdock, Courtney	3	Plenary	Mon., 12 Aug	11:30
Mylonakis, Eleftherios	182	Cross Divisional Symposium	Thurs., 15 Aug	15:00
Nakai, Madoka	146	Viruses 3	Wed., 14 Aug	16:45
Nam, Sunghee	F-20	Poster Session	Wed., 14 Aug	10:30 - 15:30
Narva, Kenneth E.	B-14	Poster Session	Wed., 14 Aug	10:30 - 15:30
Nermuť, Jiří	N-7	Poster Session	Wed., 14 Aug	10:30 - 15:30
Nielsen-LeRoux, Christina	65	Bacteria 2	Tues., 13 Aug	8:30
Niu, Jinzhi	123 STU	Diseases of Beneficial Invertebrates 2	Wed., 14 Aug	14:45
Noune, Christopher	V-19 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Ormskirk, M. Marsha	141 STU	Microbial Control 2	Wed., 14 Aug	17:30
Orozco, Rousel A.	73 STU	Nematodes 2	Tues., 13 Aug	8:45
Ortiz-Urquiza, Almudena	48	Fungi 2	Mon., 12 Aug	17:45
Padilla-Guerrero, Israel Enrique	28	Fungi 1	Mon., 12 Aug	14:45
Park, Hyun-Woo	66	Bacteria 2	Tues., 13 Aug	8:45
Park, Kwan	MC-11	Poster Session	Wed., 14 Aug	10:30 - 15:30

Pauron, David	154	Bacteria 3	Wed., 14 Aug	17:15
Pedrini, Nicolás	F-21	Poster Session	Wed., 14 Aug	10:30 - 15:30
Pell, Judith K.	181	Cross Divisional Symposium	Thurs., 15 Aug	14:30
Perera, Omaththage P.	80	Viruses 1	Tues., 13 Aug	11:00
Polanczyk, Ricardo A.	MC-12	Poster Session	Wed., 14 Aug	10:30 - 15:30
Polanczyk, Ricardo A.	MC-13	Poster Session	Wed., 14 Aug	10:30 - 15:30
Polanczyk, Ricardo A.	MC-14	Poster Session	Wed., 14 Aug	10:30 - 15:30
Popham, Holly	V-5	Poster Session	Wed., 14 Aug	10:30 - 15:30
Poppinga, Lena	90	Diseases of Beneficial Invertebrates 1	Tues., 13 Aug	11:30
Puckett, Gwyn L.	103 STU	Microsporidia Symposium	Wed., 14 Aug	9:10
Půža, Vladimír	178	Nematodes 3	Thurs., 15 Aug	9:00
Qin, Yuqi	49	Fungi 2	Mon., 12 Aug	18:00
Qiu, Lei	171 STU	Fungi 4	Thurs., 15 Aug	8:30
Rabalski, Lukasz	V-20 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Rangel, Drauzio E.N.	F-22	Poster Session	Wed., 14 Aug	10:30 - 15:30
Rehner, Stephen A.	97	Fungi Symposium	Wed., 14 Aug	9:10
Rivera, Monique J.	12 STU	Nematodes 1	Mon., 11 Aug	14:45
Roberts, Donald W.	96	Fungi Symposium	Wed., 14 Aug	8:50
Rohrmann, George F.	85	Viruses 1	Tues., 13 Aug	12:15
Rowley, Daniel L.	V-13	Poster Session	Wed., 14 Aug	10:30 - 15:30
Saito, Taro	186	Microbial Control 3	Thurs., 15 Aug	14:15
Saito, Yasumasa	V-21 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Samuel, Buck S.	13	Nematodes 1	Mon., 11 Aug	15:00
Schneider, Diana	166 STU	Viruses 4	Thurs., 15 Aug	9:15
Schoeters, Floris	104 STU	Microsporidia Symposium	Wed., 14 Aug	9:30
Schwartz, Jean-Louis	158	Bacteria Symposium	Thurs., 15 Aug	8:30
Sekiguchi, Minori	V-22 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Serna-Sarriás, M.J.	F-23	Poster Session	Wed., 14 Aug	10:30 - 15:30
Serrano, Amaya	163 STU	Viruses 4	Thurs., 15 Aug	8:30
Shapiro-Ilan, David I.	14	Nematodes 1	Mon., 11 Aug	15:15
Shapiro-Ilan, David I.	117	Cross Divisional Symposium	Wed., 14 Aug	15:00
Sharififar, Shahram	N-14 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Shi, Lian Gen	F-24	Poster Session	Wed., 14 Aug	10:30 - 15:30
Shi, Lian Gen	F-25	Poster Session	Wed., 14 Aug	10:30 - 15:30
Shu, Changlong	67	Bacteria 2	Tues., 13 Aug	9:00
Shu, Changlong	B-15	Poster Session	Wed., 14 Aug	10:30 - 15:30
Simi, Lucas D.	N-8	Poster Session	Wed., 14 Aug	10:30 - 15:30
Simi, Lucas D.	N-9	Poster Session	Wed., 14 Aug	10:30 - 15:30
Slavicek, James M.	191	Viruses 5	Thurs., 15 Aug	14:15
Sparks, Michael E.	MC-16	Poster Session	Wed., 14 Aug	10:30 - 15:30
Spigler, Madeline	F-26	Poster Session	Wed., 14 Aug	10:30 - 15:30
St. Leger, Raymond	98	Fungi Symposium	Wed., 14 Aug	9:30
Steele, Thomas	102 STU	Microsporidia Symposium	Wed., 14 Aug	8:50
Stentiford, Grant D.	DB-2	Poster Session	Wed., 14 Aug	10:30 - 15:30

Stentiford, Grant D.	DB-3	Poster Session	Wed., 14 Aug	10:30 - 15:30
Stentiford, Grant D.	51	Diseases of Beneficial Invertebrates Symposium	Tues., 13 Aug	8:20
Stephan, Dietrich	B-16	Poster Session	Wed., 14 Aug	10:30 - 15:30
Stephan, Dietrich	F-27	Poster Session	Wed., 14 Aug	10:30 - 15:30
Stevens, Glen	15	Nematodes 1	Mon., 11 Aug	15:30
Stevens, Glen	N-10	Poster Session	Wed., 14 Aug	10:30 - 15:30
Strange, James P.	DB-4	Poster Session	Wed., 14 Aug	10:30 - 15:30
Sun, Xiulian	147	Viruses 3	Wed., 14 Aug	17:15
Surendra Dara	187	Microbial Control 3	Thurs., 15 Aug	14:30
Surendra Dara	F-28	Poster Session	Wed., 14 Aug	10:30 - 15:30
Surlis, Carla	DB-7 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Swamy, Mahadeva	B-17 STU	Poster Session	Wed., 14 Aug	10:30 - 15:30
Tartar, Aurélien	29	Fungi 1	Mon., 12 Aug	15:00
Theilmann, David A.	110	Viruses 2	Wed., 14 Aug	9:30
Traver, Brenna E.	91	Diseases of Beneficial Invertebrates 1	Tues., 13 Aug	11:45
Tung, Hsuan	111 STU	Viruses 2	Wed., 14 Aug	9:45
Tweten, Rodney	160	Bacteria Symposium	Thurs., 15 Aug	9:30
Ugine, Todd A.	MC-17	Poster Session	Wed., 14 Aug	10:30 - 15:30
Ulug, Derya	16 STU	Nematodes 1	Mon., 11 Aug	15:45
Uribe-Lorío, L	B-19	Poster Session	Wed., 14 Aug	10:30 - 15:30
Uribe, Lidieth	B-18	Poster Session	Wed., 14 Aug	10:30 - 15:30
Vacari, Alessandra Marieli	MC-18	Poster Session	Wed., 14 Aug	10:30 - 15:30
Valicente, Fernando	68	Bacteria 2	Tues., 13 Aug	9:15
Valicente, Fernando	69	Bacteria 2	Tues., 13 Aug	9:30
Valicente, Fernando	V-14	Poster Session	Wed., 14 Aug	10:30 - 15:30
van Frankenhuyzen, Kees	23	Bacteria 1	Mon., 12 Aug	15:30
van Oers, Monique M.	112	Viruses 2	Wed., 14 Aug	10:00
Vandenberg, John	62	Fungi 3	Tues., 13 Aug	9:45
Vandenberg, John	F-29	Poster Session	Wed., 14 Aug	10:30 - 15:30
Veiga, Ana Carolina Pires	MC-19	Poster Session	Wed., 14 Aug	10:30 - 15:30
Velez, Ana Maria	155 STU	Bacteria 3	Wed., 14 Aug	17:30
Vijayendran, Diveena	83 STU	Viruses 1	Tues., 13 Aug	11:45
Vlak, Just	32	Viruses Symposium	Mon., 12 Aug	16:30
Vojvodic, Svjetlana	183	Cross Divisional Symposium	Thurs., 15 Aug	15:30
Volkoff, Anne-Nathalie	36	Viruses Symposium	Mon., 12 Aug	18:30
Wang, Aisuo	74	Nematodes 2	Tues., 13 Aug	9:00
Wang, Jie	173 STU	Fungi 4	Thurs., 15 Aug	8:45
Waterfield, Nick R.	132	Nematode/NEMASYM Symposium	Wed., 14 Aug	16:30
Wenzel, Inajá. M	MC-20	Poster Session	Wed., 14 Aug	10:30 - 15:30
Wenzel, Inajá. M	MC-21	Poster Session	Wed., 14 Aug	10:30 - 15:30
West, Lee	MC-22	Poster Session	Wed., 14 Aug	10:30 - 15:30
Williams, Bryony	127	Microsporidia 1	Wed., 14 Aug	13:45
Wiredu Boakye, Dominic	105 STU	Microsporidia Symposium	Wed., 14 Aug	9:50
Wollenberg, Amanda C.	70	Nematodes 2	Tues., 13 Aug	8:00

Wraight, Stephen P.	100	Fungi Symposium	Wed., 14 Aug	10:10
Wu, Tzong-Yuan	84	Viruses 1	Tues., 13 Aug	12:00
Yan, Jianping	B-20	Poster Session	Wed., 14 Aug	10:30 - 15:30
Yang, Kai	113	Viruses 2	Wed., 14 Aug	10:15
Ying, Sheng-Hua	30	Fungi 1	Mon., 12 Aug	15:15
Yu, Ziniu	N-11	Poster Session	Wed., 14 Aug	10:30 - 15:30
Zanardo, Ana Beatriz R.	F-30	Poster Session	Wed., 14 Aug	10:30 - 15:30
Zhang, Lili	41	Microbial Control 1	Mon., 12 Aug	17:30
Zhao, Ni	B-21	Poster Session	Wed., 14 Aug	10:30 - 15:30
Zheng, Congyi	V-15	Poster Session	Wed., 14 Aug	10:30 - 15:30
Zhou, Yin	148	Viruses 3	Wed., 14 Aug	17:15
Zhou, Zishan	24 STU	Bacteria 1	Mon., 12 Aug	15:45

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