38th ANNUAL MEETING
Society for
INVERTEBRATE PATHOLOGY

PROGRAM and ABSTRACTS

7-11 August 2005
Anchorage, Alaska
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Society for Invertebrate Pathology

President
Just Vlak
Wageningen University, Laboratory of Virology,
Binnenhaven 11, Wageningen 6709 PD, The Netherlands
Phone / Fax: +31 31 748 3090 / +31 31 748 4820
Email: just.vlak@wur.nl

Vice President
Wendy Gelernter
Pace Consulting, 1267 Diamond St, San Diego, CA 92109, USA
Phone / Fax: (858) 272-9897 / (858) 483-6349
Email: gelernt@paceturf.org

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Suzanne Thiem
Michigan State Univ, Dept of Entomology, S-18A Plant Biology,
East Lansing, MI 48824, USA
Phone / Fax: (517) 432-1713 / (517) 353-5598
Email: smthiem@msu.edu

Secretary
Peter Krell
University of Guelph, Dept of Molecular and Cellular Biology,
50 Stone Road East, Guelph, ON N1G 2W1, Canada
Phone / Fax: (519) 824 4120 x53368 / (519) 837 1802
Email: pkrell@uoguelph.ca

Past President
Harry Kaya
University of California, Dept. of Nematology,
One Shields Avenue, Davis, CA 95616-8668, USA
Phone / Fax: (530) 752-1051 / (530) 752-5809
Email: hkkaya@ucdavis.edu

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Chair: Kelli Hoover
Co-Chair: Diana Cox-Foster
Program: Bryony Bonning
Local Arrangements: David Smith

Please join the Organizing Committee and SIP in gratefully acknowledging the invaluable contributions and efforts of the following:

Vincent D’Amico
Mary Barbarchek, Liwang Cui, Lerry Lacey
Jim Slavicek, Lee Solter, Suzanne Thiem, Rich Humber
David Smith
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\textbf{STU} indicates papers being judged for graduate student presentation awards
\textbf{129} indicates abstract number for ORAL presentation
\textbf{B-11} indicates abstract number for POSTER presentation
**SUNDAY - 7 August**

8:30–5:00  SIP Council Meeting  Canary Room, HC
1:00–7:00  Registration  Commons Grand Hall
6:00–9:00  Mixer  Cuddy Center

**MONDAY - 8 August**

7:00–9:00  Registration  Cuddy Center

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**Opening Ceremonies and SIP Founders’ Memorial Lecture**

Monday, 8:00-10:00. Wendy Williamson Aud.

**Opening Ceremonies**

Kelli Hoover, Chair, Organizing Committee
Just Vlak, President, SIP

**Founders’ Memorial Lecture**

Dudley Pinnock, Chair, Founders’ Lecture Committee
Honoree:  **ELIZABETH CANNING**
Lecturer:  **JAMES BECNEL**

Expanding frontiers for Microsporidia: A tribute to Professor Elizabeth U. Canning

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**Plenary Symposium**  Monday, 10:30–12:30. Wendy Williamson Aud.

**Invertebrate pathogens: Evolution and impact**

Organizers: Bryony Bonning, Diana Cox-Foster.
Moderator: Diana Cox-Foster.

10:30  **1** One step ahead of emerging crustacean viruses.  
CF Leo, Natl Taiwan Univ, Taiwan

11:00  **2** Molecular adaptations for pathogenicity in
Metarhizium anisopliae.  
R St Leiper, Univ of Maryland, USA

11:30  **3** All models are wrong, but some models are useful: Using mechanistic models to understand insect pathogens.  
G Dwyer, Univ of Chicago, IL, USA

12:00  **4** Invertebrates as a source of emerging human pathogens.  
Gffrench-Constant, Univ of Bath, UK

12:30–2:00  **LUNCH**  Cuddy Center

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**Symposium (Cross Divisional)**  Monday, 2:00-4:00. BEB 101

**Diseases of marine invertebrates**

Organizer: Carolyn Friedman.

2:00  **5** Hematodinium spp.: Emergent pathogens for several commercial species of marine crustaceans.  
T Meyers, Alaska Dept of Fish and Game, Juneau, Alaska, USA

3:00  **6** Herpesviruses infecting bivalves.  T Renault, IFREMER Lab de Genétique, Aquaculture et Pathologie, La Tremblade, France

3:15  **7** Characterization of *Perkinsus* spp. and oyster herpes-like virus found in oysters collected in China, Japan and Korea.  
K Reece, Virginia Institute of Marine Science, Gloucester, Virginia, USA

3:30  **8** Withering Syndrome, a rickettsial disease of abalone, *Haliotis* spp.  
C Friedman, Univ of Washington, Seattle, WA, USA

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**Contributed Papers**  Monday, 2:00-4:00. BEB 110

**FUNGI 1**

Moderators: Jarrod Leland and Denny Bruck.

2:00  **9** Susceptibility of four native lady beetle species to *Beauveria bassiana*.  
TE Cottrell, DJ Shapiro-Han, USDA, Agric Res Service, Byron, GA, USA

2:15  **10** Reduced susceptibility of over-wintering ladybirds to *Beauveria bassiana*.  
H Roy, E Ormond, M Majerus. 1Dept of Life Sciences, Anglia Polytechnic Univ, and 2Dept of Genetics, Univ of Cambridge, Cambridge, UK

2:30  **11** Effect of in vivo passage of *Beauveria bassiana* through aphid versus non-aphid hosts on the relative virulence towards two cereal aphid species (*Homoptera: Aphididae*).  
JL Hatting, ARC-Small Grain Instit, Bethlehem, South Africa.

2:45  **12** Changes in virulence to Colorado potato beetles of *Beauveria bassiana* GHA isolates recovered from sprayed fields one to four years post application.  
LA Castrillo, MH Griggs, E Groden, SL Annis, PK Mishra, JD Vandenbergh. 1Dept of Entomology, Cornell Univ, 2USDA-ARS, US Plant, Soil and Nutrition Lab, Ithaca, NY, 3Dept of Biological Sciences, Univ of Maine, Orono, ME, USA

3:00  **13** Identification and characterization of genes responsible for pathogenicity of *Beauveria bassiana* towards the coffee berry borer.  
JG Mantilla, AL Gaitan, CE Gongora, 1Dept of Plant Pathology, CENICAFE, Chinchina, Caldas, Colombia

3:15  **14** Germination polarity of conidia and its correlation with pathogenicity of *Beauveria bassiana* isolates.  
R Talaei-hassanlou1,2, A Kharaizi-pakdel1,3, MS Goettel1, S Little, J Mozaffari. 1Dept of Plant Protection, College of Agriculture, Univ of Tehran, Karaj, Iran, 2Lethbridge Research Centre, Lethbridge, Alberta, Canada, 3Dept of Genetics, Seed and Plant Improvement Institute, Karaj, Iran

3:30  **15** Virulence and fitness of the fungal pathogen *Entomophaga maimaiga* in its host *Lymantria dispar*, for pathogen and host strains originating from Asia, Europe and North America.  
C Nielsen, M Keena, AE Hajek. 1Dept of Entomology, Cornell Univ, Ithaca, NY, USA, 2USDA Forest Service, Northeastern Research Station Hamden, CT, USA

3:45  **16** Growth characteristics and virulence of insect pathogenic fungi at low temperatures.  
L Hjeljord, 1L Klingn, 2Norwegian Univ of Life Sciences, 3The Norwegian Crop Research Institute, Hogskelev. Aas, Norway
BACTERIA 1

Moderator: Alejandra Bravo.

2:00 17 Characterization of the cadherin protein from *Lymantria dispar* as Cry1A toxin receptor. JL Jurat-Fuentes1, AP Valaitis2, MJ Adang1, 1Dept of Entomology and 2Biochemistry & Molecular Biology, Univ of Georgia, Athens, GA, USA, 2, USA/DA For Serv, Delaware, OH, USA

2:15 18 Mapping the binding epitopes for cadherin-like receptor (BT-R1) on *Bacillus thuringiensis* Cry1Aa toxin. X Liu, DH Dean, Dept of Biochemistry, Ohio State Univ, Columbus, OH, USA

2:30 19 A detergent-like mode of action of the Bt toxin Cry1Aa in insect brush border membrane vesicles. M Kirouac, V Vachon, D Quievy, J-L Schwartz, R Laprade, Groupe d’étude des protéines membranaires, Univ de Montréal, Centre Ville Station, Montreal, Quebec, and Biocontrol Network, Canada

2:45 20 Protease inhibitors fail to prevent pore formation by the activated *Bacillus thuringiensis* toxin Cry1Aa in insect brush border membrane vesicles. M Kirouac, V Vachon, D Quievy, J-L Schwartz, R Laprade, Groupe d’étude des protéines membranaires, Univ de Montréal, Centre Ville Station, Montreal, Quebec, and Biocontrol Network, Canada

3:00 21 Differential effects of ionic strength and pH on the pore-forming activity of *Bacillus thuringiensis* insecticidal toxins. M Fortier, M Kirouac, V Vachon, O Peyronnet, J-L Schwartz, R Laprade, Groupe d’étude des protéines membranaires, Univ de Montréal, Centre Ville Station, Montreal, Quebec, and Biocontrol Network, Canada

3:15 22 Mode of action of *Bacillus thuringiensis* insecticidal toxin Cry9Ca: Effect of the physico-chemical microenvironment on pore formation in *Manduca sexta* intestinal membranes. J-F Brunet1, V Vachon1, M Marsolais1, J van Rie2, J-L Schwartz2, R Laprade2, 1Groupe d’étude des protéines membranaires, Univ de Montréal, Centre Ville Station, Montreal, Quebec, and Biocontrol Network, Canada

3:30 23 Directed mutagenesis of conserved aromatic residues in helix 7 critical for larvicidal activity of the *Bacillus thuringiensis* Cry4Ba toxin. K Tiewsiri1, Vachon1, Diptima1, Dept of Biochemistry, Univ of Exeter, Hatherly College, Sault Sainte Marie, Ont, Canada, 2School of Biological and Chemical Sciences, Univ of Exeter, Hatherly Laboratories, Devon, UK

3:45 24 Mutagenic analysis of the transmembrane helix 5 of the *Bacillus thuringiensis* Cry4Ba toxin reveals a crucial role in larvicidal activity for *Asn-183*. S Likitvivatanavong1, C Angusathanasombat1, Lab of Molec Biophys and Struct Biochem, Institute of Molecular Biology and Genetics, Mahidol Univ, Salaya Campus, Nakornpathom, Thailand

4:00–4:20 BREAK

SYMPOSIUM (Div. of Microbial Control) Monday, 4:20–6:20, BEB 101

Use of pathogens against insect pests

Organizers/Moderators: Maureen O’Callaghan, Travis Glare.

4:20 25 Eradication of invasive lepidopteran pests with *Foray*, R Fusco, A Rath, Valens BioSciences Corp. USA and Canada

4:44 26 Assessing short-term health effects of *Bacillus thuringiensis* applied during insect control programs. D Levin, Univ of Victoria, British Columbia, Canada

5:08 27 Use of pathogens against incursion pests in New Zealand. T Glare, IR Gear, AgResearch, Lincoln, New Zealand

5:32 28 Development of fungal bands to assist in eradication of Asian longhorned beetle, *Anoplophora glabripennis*, in the U.S. A Hauck1, JR Reilly1, T Dubois1, M Smith2, L Bauer1, Z Li3, 1Dept Entomology, Cornell Univ NY, 2USDA Newark, DE, 3USDA Michigan, 4Dept Forestry, Anhui Agricultural Univ, China

5:56 29 Varroa mite control with fungal pathogens: Will this little piggy get to market? R James, USDA-ARS Bee Lab, Utah State Univ, Logan, UT, USA

VIRUSES 1

Moderator: Basil Arif.

4:20 30 Pathogen diversity and the efficacy of virus insecticides. JS Cory1, DJ Hodgson1, EM Redman2, 1Ecology and Biocontrol Group, NERC Centre for Ecology and Hydrology, Oxford, UK, 2Inst for Animal Health, Pirbright Laboratory, Woking, Surrey, UK, 3Ecology and Evolution Research Group, School of Biology, Univ of Leeds, UK

4:50 32 Investigating the genetic parameters that affect virus transmission. EL King1, RS Hails1, RD Possee1, LA King1, 1School of Molecular and Biological Sciences1, Oxford Brookes Univ, Oxford, 2NERC Institute of Virology and Environmental Microbiology (CEH), Oxford, UK

5:05 33 Biological and molecular characterization of iranian-caucasian isolates of *Cydia pomonella* granulovirus (CpGV). S Sayed1,2, M Rezapapanah1, S Shojaei-Estrabragh1,4 JA Jaleh1, 1Lab of Biotechnological Crop Protection, Agricultural Service Center Palatinate, Neustadt/Wstr., Germany, 2Dept of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo Univ, Egypt, 3Biocontrol Research Dept, Plant Pests and Diseases Research Institute, Tehran, Iran, 4National Research Center of Genetic Engineering & Biotechnology, Tehran, Iran

5:20 34 Enhancement in activity of *Turkish SpliNPV-B* to *Spodoptera littoralis* Boisd. (Lepidoptera:Nocuidae) by an optical brightener. U Toprak1, O Gurkan, Univ of Ankara, Faculty of Agriculture, Dept of Plant Protection, Ankara, Turkey

5:35 35 Nutritional self-medication by insects in response to protein costs of virus resistance. KP Lee1, JS Cory1, K Wilson1, D Rauenheimer1, SJ Simpson1, 1Dept of Zoology, Univ of Oxford, UK, 2Dept of Biological Sciences, Institute of Environmental and Natural Sciences, Univ of Lancaster, UK, 3Molecular Ecology and Biocontrol Group, NERC Centre for Ecology and Hydrology, Oxford, UK

5:50 36 Disruption of climbing behavior prior to death of gypsy moth (*Lymantria dispar*) larvae infected with egt-deletion constructs of *LdNPV*. M Grove1, B Reed2, AD Jones3, N Hayes-Plazolles1, J Slavicek1, K Hoover1, 1The Pennsylvania State Univ, Dept of Entomology, PA, USA, 2USDA Forest Service, Delaware, OH, USA
4:20  **Competition between the microsporidia *Nosema* *lymantriae* and *Vairimorpha* sp. parasitizing *Lymantria dispar* larvae: The importance of timing for successful establishment and horizontal transmission of infection.**

D Pilarska¹, LF Solter², A Linde³, M Kereselidze⁴, G Hoch⁴, ¹Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria, ²Center for Economic Entomology, Illinois Natl Hist Survey, Champaign, IL, USA, ³Dept of Forestry, Univ of Applied Sciences, Eberswalde, Germany, ⁴V. Guisasalashvili Institute of Mountain Forestry, Academy of Sciences, Tbilisi, Republic of Georgia, ⁵Dept of Forest and Soil Sciences, BOKU Univ of Natural Resources and Applied Life Sciences, Vienna, Austria

5:05  **Microsporidia in *Hippodamia convergens* (Guérin-Méneville) used for biological control in agroecosystems.**

S Bjornson, Dept of Biology, Saint Mary’s University, Halifax, Nova Scotia, Canada

5:20  **Do *Johenrea locustae* and *Paranosema locustae* represent two different developmental sequences of the same species?**

YY Sokolova¹, CE Lange², YS Tokarev³, JJ Fuxa¹, ¹Dept of Entomology, Louisiana State Univ AgCenter, Baton Rouge, Louisiana, USA, ²Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia, ³Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria, ⁴Institute of Mountain Forestry, Academy of Sciences, Tbilisi, Republic of Georgia, ⁵Dept of Parasitological Studies, La Plata, Argentina, ⁶Institute for Plant Protection, Russian Academy of Agricultural Sciences, St. Petersburg, Russia.

5:35  **Microsporidian parasites in freshwater snails.**

HE McClveymont¹, AM Dunn¹, RS Terry², D Rollin², DTJ Littlewood³, JE Smith³, ¹School of Biology, Univ of Leeds, UK, ²The Natural History Museum, London, UK

5:50  **PruP2, a transpositional repressor, regulates sexual development in the malaria parasite *Plasmodium falciparum.***

J Li¹, Q Fan¹, L Cui¹, ¹Dept of Entomology, The Pennsylvania State Univ, University Park, PA, USA, ²Dept of BioScience and Technology, Dalian Univ of Technology, Liaoning, China

6:05  **Analysis of *Gregarines niphandrodes* mitochondria.**

MA Toso, CK Omost, School of Biological Sciences, Washington State Univ, Pullman, WA

**Contributed Papers Monday, 4:20-6:20. BEB 111**

**MICROSPORIDIA AND PROTOZOA**

Moderator: Andreas Linde.
**TUESDAY AM**

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**TUESDAY - 9 August**

6:30 5K Fun Run / Walk  
Behm Lake, near dorms

Symposium (Cross-Divisional)  
Tuesday, 8:00-10:00. BEB 101

**Transmission of invertebrate pathogens**  
Organizer/Moderator: Rosalind R. James.

8:00 55 The evolution of virulence and transmission of disease. P Agnew, CNRS/IRD, Montpellier, France

8:24 56 Factors affecting transmission of fungal pathogens of aphids. D Steinkraus, Univ of Arkansas, Fayetteville, AR, USA

8:48 57 Consideration of vertically transmitted microsporidia for biological control. L Solter, Illinois Nati Hist Survey, Urbana, IL, USA

9:12 58 Transmission of viruses to mosquito larvae mediated by divalent cations. J Beemel, USDA-ARS, Gainesville, Florida, USA

9:36 59 Effect of mono- and poly-gyne social forms on transmission and spread of microsporidia in fire ant populations. D Qi, USDA-ARS, Gainesville, Florida, USA

Symposium (Division of Fungi)  
Tuesday, 8:00-10:00. BEB 111

**Emerging genomics of fungal entomopathogens**  
Organizers/Moderators: Nemat O. Keyhani and Paresh Shah.

8:00 60 Generation of a robust EST dataset for Beauveria bassiana. F-M Cho, NO Keyhani, Dept of Microbiology and Cell Science, Univ of Florida, Gainesville, FL, USA

8:30 61 Developmental and transcriptional responses to host and non host cuticles by the specific locust pathogen Metarhizium anisopliae var. acridum. R St Leger, Dept of Entomology, Univ of Maryland, MD, USA

9:00 62 Linking ESTs to gene function and secondary metabolite discovery in Metarhizium anisopliae. A Churchill, Dept of Plant Pathology, Cornell Univ, Ithaca, NY, USA

9:30 63 Sense and sensibility in the genomic age. R Humber, USDA-Cornell, New York, USA

**Contributed Papers**  
Tuesday, 8:00-10:00. BEB 117

**NEMATODES AND SYMBIOTIC BACTERIA**  
Moderator: Mary Barbercheck.

8:00 64 Insecticidal toxins from Photorhabdus bacteria. R Frenche-Constant, N Waterfield, A Dowling, G Yang, Dept of Biology and Biochemistry, University of Bath, UK

8:15 65 Mixing and matching of toxin complex proteins. T Hey, S Bevan, A Schleper, P Birkhold, S Burton, T Meade, D Merlo, J Sheets, R Thompson, H Moon, Dow AgroSciences, Indianapolis, IN, USA

8:30 66 Novel toxin complex constructions. T Hey, C Cai, A Woosley, S Burton, J Sheets, B Waldman, H Moon, T Meade, D Merlo, Dow AgroSciences, Indianapolis, IN, USA

8:45 67 The characterisation of the structure of Xenorhabdus insecticidal toxin component XptA1. SC Lee, S McPhie, A Rodger, DI Roper, J Henderson, M Sergeant, JAW Morgan, ¹Warwick HRI, Univ of Warwick, Wellesbourne, Warwick, UK, ²Dept of Biological Sciences, and ³Dept of Chemistry, Univ of Warwick, Coventry, UK, ⁴Univ of Nottingham, UK, ⁵School of Biological Sciences, Coventry Univ, UK

9:00 68 The hemolysin alpha-xenorhabdolysin secreted by pathogenic enterobacteria belongs to a new family of cytotoxins and triggers apoptosis. F Vigneux, A Givaudan, PA Girard, C Ribeiro, S Baghdiguian, M Brehelin, ¹Laboratoire d'Ecologie Microbiene des Insectes Interactions Hotes-Pathogene, INRA-Univ de Montpellier II, France, ²Institut des Sciences de l'Evolution, Univ de Montpellier II, France

9:15 69 Effect of harvest time and culture conditions on the morphology and ultrastructure of the bacterial receptacle in Steinernema carpopcapae (Nematoda: Steinernematidae). SP Stock, Y Vega, Dept of Plant Sciences, ²Dept of Entomology, University of Arizona. Tucson, USA

8:00 70 Genetic and molecular analysis of infectious juvenile longevity in the entomopathogenic nematode Heterorhabditis bacteriophora. SK Sandhu, PS Grewal, Dept of Entomology, The Ohio State Univ, OARDC, Wooster, OH, USA

9:45 71 Characterization of surface coat proteins from Steinernema glaseri that suppress immune responses in Oriental beetle larvae. X Li, RS Cowles, E Cowles, R Gaugler, AD Jones, DL Cox-Foster, ¹Dept of Entomology, and ²Dept of Chemistry, The Pennsylvania State Univ, PA, USA, ³Valley Laboratory, The Connecticut Agricultural Experiment Station, Windsor, CT, USA, ⁴Dept of Biology, Eastern Connecticut State Univ, Willimantic, CT, USA, ⁵Dept of Entomology, The Rutgers Univ, New Brunswick, NJ, USA

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**Contributed Papers**  
Tuesday, 8:00-10:00. BEB 110

**VIRUSES 2**  
Moderator: Doreen Winstanley.

8:00 72 A cell culture system and infectious clone for the study of Rhopalosiphum padi virus (Dicistrovidiae). S Boyapalle, R Becker, WA Miller, ¹BC Bonning, ²Depts of Entomology and Plant Pathology, Iowa State Univ, Ames, IA, USA

8:15 73 Baculovirus expression of Rhopalosiphum padi virus (Dicistrovidiae). S Boyapalle, R Becker, WA Miller, ¹BC Bonning, ²Depts of Entomology and Plant Pathology, Iowa State Univ, Ames, IA, USA

8:30 74 Characterization of a new virus isolated from the rosy apple aphid, Dysaphis plantaginea. N Naish, E Ryabov, D Winstanley, Warwick HRI, Univ of Warwick, Wellesbourne, UK

8:45 75 Comparative viral RNA loads in deformed wing virus infected Apis mellifera L. and its ectoparasite Varroa destructor. D Tentscheva, L Gauthier, B Dainat, F Cousserans, ME Colin, M Bergeon, Laboratoire de Pathologie Comparée des Invertébrés EPHE, Biologie Intégrative et Virologie des Insectes, Univ Montpellier II, France

9:00 76 Analysis of the poly(A) polymerase encoded by the entomopoxvirus, AMEV. MN Becker, TM Todd, RW Moyer, Dept of Molecular Genetics and Microbiology, College of Medicine, Univ of Florida, Gainesville, FL, USA

9:15 77 Virus tropisms is controlled by insect parovirus promoters. P Trijsens, J Zelei, M El-Far, G Fédière, INRS-Institut Armand-Frappier, Laval, QC, Canada
TUESDAY AM

9:30  
STU

78 Analysis of the immediate early me53 gene from the baculovirus AcMNPV. J de Jong1, DA Theilmann2, BM Arif3, PJ Krell1. 1Dept of Molecular and Cellular Biology, Univ of Guelph, ON, Canada, 2Agriculture and Agri-food Canada, Pacific Agri-food Centre, Summerland, BC, Canada, 3Canadian Forest Service, Great Lakes Forestry Research Centre, Sault Ste. Marie, ON, Canada

9:45  
STU

79 Characterization of Cylia pomenella granulovirus metalloproteinase. EM Kemp, SL Hilton, D Winstanley, Warwick HRI, Wellesbourne, Warwick, UK

10:00–10:20 BREAK BEB Lobby

SYMPOSIUM (Virus Division)  Tuesday, 10:20–12:20. BEB 101

Polydnaviruses and Ascoviruses
Organizers/Moderators: Peter Krell and Michel Cusson.

10:20  
STU

80 A polydnavirus paradox: Cophylogeny and mosaic genomes. J Whitfield, Univ of Illinois at Urbana-Champaign, IL, USA

10:50  
STU

81 Polydnavirus genomics: Form and function of mutualistic insect viruses from parasitic wasps. B Webb, Univ of Kentucky, Lexington, Kentucky, USA

11:20  
STU

82 Inferring evolution through the biology of ascoviruses. X-W Cheng, Miami Univ, Oxford, Ohio, USA

11:50  
STU

83 The biology of polydnaviruses and their interactions with insect hosts. M Beckage, Univ of California, Riverside, CA, USA

Contributed Papers  Tuesday, 10:20–11:35. BEB 110

ALGAE, OTHER

Moderator: James Becnel.

10:20  
STU

84 Women pioneers of invertebrate cell culture. Karl Maramorosch, Dept of Entomology, Rutgers Univ, New Brunswick, New Jersey, USA

10:35  
STU

85 Genomics approaches to insect-pathogen relationships in the spuce budworm, Choristoneura fumiferana. Q Feng, 1T Ladd1, S Zheng1,2, L Li1, D Zhang1,2, D Buhrers1, PJ Krell1, BM Arif3, A Retnakaran1. 1Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada, 2Dept of Microbiology, Univ of Guelph, ON, Canada

10:50  
STU

86 Development and pathway of infection of the entomopathogenic alga Helicospordinium (Chlorophyta: Trebouxiophyceae). Y-U Bläské-Lierze, DG Boucias, Entomology and Nematology Dept, Univ of Florida, Gainesville, FL, USA

11:05  
STU

87 Helicospordinium sp. infection in mosquito larvae. TM Conklin1, V-U Bläské1, JJ Becnel2, DG Boucias2. 1Dept of Entomology and Nematology, Univ of Florida, Gainesville FL, USA, 2USDA, CMAVE, Gainesville, FL, USA

11:20  
STU

88 Identification of genes transcribed by Moracella osloensis in slug Deroceras reticulatum using selective capture of transcribed sequences. R Ap, S Sreevatsan, P Grewal, Dept of Entomology, OARDC, The Ohio State Univ, OH, USA

TUESDAY AM

POSTERS – 1

Tuesday, 10:20-12:20. BEB Lobby, 1st and 2nd floor

POSTERS should be displayed from Monday UNTIL NO LATER THAN 1:00 pm, THURSDAY

Fungi

F-1 Characteristics and phylogenetic classification of Cordyceps and its allies, Entomopathogenic fungi. S- H Nam1, I-P Hong1, J-S Hwang1, S-B Hong2, S-D Ji1, S-W Kang3, M-S Han4. 1Dept of Agricultural Biology, and 2National Institute of Agricultural Biotechnology, National Institute of Agricultural Science Technology, RDA, Suwon, Korea, 3College of Agriculture and Life Sciences, Kyungpook National Univ, Daegu, Korea

F-2 RAPD analysis of isolates of Beauveria bassiana, a pathogenic fungus to the silkworm, Bombyx mori L. L Shi, J Jin, College of Animal Sciences, Zhejiang Univ, Hangzhou, PR China

F-3 Characterization of Beauveria bassiana isolates based on ITS and TEF sequences. R Talaei-hassanlou1, A Kharaz-pakdel1, M S Goettel2, J Mozaffari3, J Bissett4. 1Dept of Plant Protection, College of Agriculture, Univ of Tehran, Karaj, Iran, 2Lethbridge Research Centre, Lethbridge, Alberta, Canada, 3Dept of Genetics, Seed and Plant Improvement Institute, Karaj, Iran, and 4ECORC, Ottawa, Ontario, Canada

F-4 Approaches to testing a biological hypothesis that host flight dominates transmission of aphid-pathogenic fungi among aphid populations. C Chen1, M-G Feng2,3. 1Institute of Microbiology, College of Life Sciences, 2Institute of Applied Entomology, College of Agriculture and Biotechnology, Zhejiang Univ, Hangzhou, Zhejiang, PR China

F-5 Effects of entomopathogenic fungus Paecilomyces fumorososeus to common white Pieris rapae crucivora. H Hiromori, D Yaginuma, N Washizu, M Kimura, Dept of Applied Entomology, Faculty of Agriculture, Shizuoka Univ, Japan

F-6 Characterization of entomopathogenic fungi of oca weevil Adioristidius tuberculatus Voss in the Andean region of Peru. J. Salazar1, V. Cañedo1, J. Alcázar1, A. Lagnauzi2. 1International Potato Center (CIP), Lima, Peru, 2The World Bank, Environmentally and Socially Sustainable Development, Washington DC, USA

F-7 Factors relating to epizootics of Hirsetella sp. in field populations of Homalodisca coagulata (Say) (Homoptera: Cicadellidae). SE Breaux1, DG Boucias1, RF Mizell III1, 2Univ of Florida, Dept of Entomology and Nematology, Gainesville, FL, USA, 3Univ of Florida, North Florida Research and Education Center Quincy, FL, USA

F-8 Growth and virulent characteristics of Verticillium lecanii (Lecaniciullum spp.) hybrid strains. D Aiuchi1, M Koike1, K Inami1, Y Baba1, M Sugimoto1. 1Dept of Agro-environmental Science, Obihiro Univ of Agriculture and Veterinary Medicine, Hokkaido, Japan, 2Okinawa Prefectural Agricultural Experiment Station, Naha, Japan

F-9 The generalist predator Anthocoris nemorum detects and avoids Beauveria bassiana: NV Meyling1, JK Pell2. 1Dept of Ecology, The Royal Veterinary and Agricultural Univ, Thorvaldsensvej, Frederiksberg, Denmark, 2Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, UK

F-10 Interactions between over-wintering seven spot ladybirds (Coccinella septempunctata) and the entomopathogenic fungus Beauveria bassiana: The 12 buckets. E Ormond1, A Thomas1, J Pell1, H Roy1. 1Dept of Life Sciences, Anglia Polytechnic Univ, Cambridge, UK
F-11  
1Shafter Research and Extension Center, Shafter, CA, 2USDA-ARS, Shafter, CA, 3Dept of Nematology, Univ of California, Davis, CA, USA

F-12  
Comparative susceptibility of *Metarhizium anisopliae* varieties *anisopliae* and *acridum* to the selective fungicide dodine. SJ Dettenmaier, DEN Rangel, EW Evans, DW Roberts. Dept of Biology, Utah State Univ, Logan, UT, USA

F-13  
Virulence of two *Metarhizium anisopliae* varieties to Mormon cricket, *Anabrus simplex*, nymphs and adults. HG Bignayan1,2,3, DEN Rangel1,2, EW Evans1, DW Roberts1. 
1Dept of Biology, Utah State Univ, Logan, UT, USA, 2Dept de Analises Clinicas, Toxicologicas e Bromatologicas, Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Univ de Sao Paulo, Ribeirao Preto, Brazil, 3Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, USDA-ARS Northern Plains Agricultural Research Lab, Sidney, MT, USA

F-14  
Are ‘stressed-out’ wireworms more susceptible to the biocontrol agent *Metarhizium anisopliae*? JT Kabalah, M Goettel1, M Isman1, E Jovel1, JH Myers1. 
1Dept of Biology, Utah State Univ, Logan, UT, USA, 2Dept de Analises Clinicas, Toxicologicas e Bromatologicas, Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Univ de Sao Paulo, Ribeirao Preto, Brazil

F-15  
Stu  
Challenges and constraints in deploying *Metarhizium anisopliae* for biocontrol of sugarbeet root maggot, *Tetanops myopaeformis*. STU  

F-16  
Observations on the interaction between biocontrol fungi, *Metarhizium* and *Bacillus*, and bacteria isolated from the rhizosphere of sugar beets. K Jung1, C Fuller-Schaefer1, K Jung1, S Jaronski1,2. 
1USDA-ARS, Northern Plains Agricultural Research Lab, Sidney, MT, USA, 2Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany

F-17  
Influence of plant rhizosphere on the abundance of entomopathogenic fungi. DR Sosa-Criónz, AMR Almeida, JJ da Silva, LC Benato, Embrapa Soja, Brazil

F-18  
Colonization of sugarbeet roots by entomopathogenic fungi. S Jaronski1,2, S Jaronski1. 
1USDA-ARS, Northern Plains Agricultural Research Lab, Sidney, MT, USA, 2Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany

F-19  
Coffee endophytes pathogenic to the coffee berry borer. F Posada, FE Vega. Insect Biocontrol Laboratory, USDA-ARS, Beltsville, Maryland

F-20  
Low likelihood of recombination between the introduced *Beauveria bassiana* strain GHA and indigenous conspecific strains based on vegetative compatibility groupings. LA Castrillo1,2, SL Anness1, E Groden1, G Miska2, JD Vandenbreg1,2. 
1Dept of Entomology, Cornell Univ, Ithaca, New York, 2Dept of Biological Sciences, Univ of Maine, Orono, ME, 3USDA-ARS, US Plant, Soil and Nutrition Lab, Tower Road, Ithaca, NY, USA

F-21  
Purification and gene cloning of a new hydrophobin-like protein that relates to thermal tolerance of aerial conidia of fungal biocontrol agents. S-H Yim1,2, M-G Feng1,2. 
1Institute of Microbiology, Coll of Life Sciences, and 2Institute of Applied Entomology, Coll of Agriculture and Biotechnology, Zhejiang Univ, Hangzhou, Zhejiang, PR China

F-22  
Toxins are overproduced in a gene disruption mutant of *Metarhizium anisopliae*. SB Krasnoff1,2, ACL Churchill1,2, JD Vandenbreg1,2, DM Gibson1. 
1Dept of Plant Pathology, Cornell Univ, Ithaca, NY, 2Boyce Thompson Institute, Ithaca, NY, 3USDA-ARS, Plant Protection Research Unit, Ithaca, NY, USA

F-23  
A study of the expression profile of pathogenicity related genes in the entomopathogenic fungus *Beauveria bassiana* on different insect cuticles. PAA Khan1,2, KU Devi1, A Reinecke2,1. 
1Dept of Botany, Andhra Univ, Visakhapatnam, India, 2Dept of Entomology, Max-Planck Institute of Chemical Ecology, Jena, Germany

F-24  
Some *Beauveria bassiana* proteinases as one of the determinants of entomopathogenicity. U. Iskandarov, A Guzalova, Institute of Microbiology, Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan

F-25  
Targeted disruption of a peptide synthetase gene in *Metarhizium anisopliae* has no effect on destruxin production or virulence against insects. Y-S Moon1,2, SB Krasnoff1,2, DM Gibson1,2, ACL Churchill1,2,3. 
1Boyce Thompson Institute, Ithaca, NY, 2Dept of Plant Pathology, Cornell Univ, Ithaca, NY, 3USDA-ARS, Plant Protection Research Unit, Ithaca, NY, USA

BACTERIA

B-1  
Vip3B1a1: A novel Vip protein from *Bacillus thuringiensis*. C Rang1, P Gil1, N Neisner1, J van Rie1, R Frutos1,2. 
1Bayer BioScience NV, Gent, Belgium, 2CIRAD, Campus International de Baillarguet, Montpellier, France

B-2  
Identification of vip genes in *Bacillus thuringiensis* strains by PCR-RFLP. CS Hernández1,2, A Boets1, J van Rie1. 
1Departament de Genètica, Univ de València, Spain, 2Bayer BioScience NV, Gent, Belgium

B-3  
Novel insecticidal proteins secreted by *Bacillus thuringiensis*. JW Pitkin, K Krasomil-Osterfeld, JA Baum, WP Donovan, AG Gao, LA Harrison, LA Casagrande, NA Biest, WP Clinton, O Ilagan, MR Walters, JM Curtis, RD Simpson, JK Roberts, Agronomic Traits, Monsanto Company, Chesterfield, MO, USA

B-4  
Antibacterial activity of *Bacillus thuringiensis* strains. GV Kalmykova, L I Burteva, Lab of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia

B-5  
Molecular cloning of novel crystal protein genes, cry3OC and s2Orf2, from a mosquitocidal *Bacillus thuringiensis* serovar *sotto* strain. A Ohushii1,2, N Wasano1, H Saiioh1, A Uemori1, M Obih1,2. 
1Graduate School of Agriculture, Kyushu Univ, Fukuoka, Japan, 2Biotechnology and Food Research Institute, Kureme, Fukuoka, Japan

B-6  
Utilisation of the Rhs core region of *te-sepC* orthologues as a degenerate system for the rapid amplification of putative insecticidal genes. MRH Hurst, NA Bockett, Biocontrol and Biosecurity, AgResearch, Lincoln, New Zealand

B-7  
FlhA flagella basal body protein influences transcription of PlcR regulated genes, protein production and virulence of *Bacillus thuringiensis*. L Bouillaut1, C Buisson1, C Nielsen-LeRoux1,2.
B-11 Functional analysis of the cadherin protein from *Heliothis virescens* as Cry1Ac receptor. JL Jurat-Fuentes1, MJ Adang1,2, Depts of Entomology and Biochemistry & Molecular Biology2, Univ of Georgia, Athens, GA, USA

B-12 CR12-MPED fragment of *Manduca sexta* Br-R9 cadherin enhances activity of Br Cry1A toxins. G Hual1, J Chen1, JL Jurat-Fuentes1, MA Abdullah1, M Adang1,2, Depts of Entomology1 & Biochemistry & Molecular Biology2, Univ of Georgia, Athens, GA, USA

B-13 Mutagenic analysis of surface-exposed loop residues critical for larvalvic activity of the *Bacillus thuringiensis* Cry4Ba toxin. T Khao khiew1, C Angus thanasomabat, Lab of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol Univ, Nakornpathom, Thailand

B-14 Studies of peptides mimicking the proposed pore-forming helices of the *Bacillus thuringiensis* Cry4Ba toxin. S Lertchawes1, C Angus thanasomabat, Lab of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol Univ, Nakornpathom, Thailand

B-15 Isolation and functional characterization of *Bacillus thuringiensis* Cry4Ba toxin-binding proteins from *Aedes aegypti* larvae. S Moosom1, C Angus thanasomabat, Lab of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol Univ, Nakornpathom, Thailand

B-16 Interaction of the Bt toxin Cry1A with lipid monolayer. S Clark1, M Pustai-Carey1, P Burkol1, Depts of Chemistry and Biochemistry, Univ of Southern Mississippi, Hattiesburg, MS, 1Dept of Biochemistry, Case Western Reserve Univ, Cleveland, OH, USA

B-17 Exposing the cryptic antibacterial potential of Cry1Ca from *Bacillus thuringiensis israelensis* by genetic manipulations. M Iske1, R Manasherob2, C Berry3, A Zaratisky4, 1Dept of Life Sciences, Ben-Gurion Univ of the Negev, Be’er-Sheva, Israel, 2Dept of Genetics, Stanford Univ, Stanford, CA, USA, 3Cardiff School of Biosciences, Cardiff Univ, Cardiff, UK

B-18 Endogenic activation of Cry2Ba toxin by camelisin from *Bacillus thuringiensis israelensis*. M Sinevitch1, S Cohen1,2, E Ben-Dov1, A Zaratisky2, R Cahan1, 1Dept of Chemical Engineering and Biotechnology, College of Judea and Samaria, Ariel, Israel, 2Dept of Life Sciences, Ben-Gurion Univ of the Negev, Be’er-Sheva, Israel

B-19 Individual characterization of the three cry1A promoters of *Bacillus thuringiensis* subsp. *israelensis*. Y Sakano1, H-W Park1, BA Federici1, 1Dept of Entomology, Univ of California, Riverside, CA, USA, 2John A. Mulrennan Sr., Public Health Entomology Research & Education Center, Florida A&M Univ, Panama City, FL, USA

B-20 Analysis of the plasmid replication origin ori165 from *Bacillus thuringiensis* subsp. *teethoniensis*. J Huang, S Guo, F Wei, M Sun, Z Yu, Coll of Life Science and Technology, Huazhong Agricultural Univ, State Key Laboratory of Agricultural Microbiology, Wuhan, PR China

B-21 Novel *Bacillus thuringiensis* strains isolated from soil samples in China. Y Meng, Z Zhang, H Qu, L Ruan, M Sun, Z Yu, Coll of Life Science and Technology, Huazhong Agricultural Univ, State Key Laboratory of Agricultural Microbiology, Wuhan, PR China

B-22 Diversity of *Bacillus thuringiensis* strains in the maize and bean phylloplane and from their respective soils in Colombia. S Jara1, P Maduell1,2, S Orduz1,2, 1Unidad de Control Biológico y Biotecnología, Corporación para Investigaciones Biológicas, Medellin, Colombia, 2Unidad de Microbiología, Facultad de Ciencias, Univ Autonoma de Barcelona, Barcelona, España, 3Facultad de Ciencias Basicas, Univ de Pamplona, Pamplona, Colombia

B-23 Characterization of selected *Bacillus thuringiensis* strains. GV Kalmykova1, LI Burtsvea1, AV Mokeva1, SF Oreshkova1, 1Lab of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia, 2Dept of Life Sciences, Ben-Gurion Univ of the Negev, Be’er-Sheva, Israel, 3Facultad de Ciencias Basicas, Univ de Pamplona, Pamplona, Colombia


B-25 Effect of *Bacillus thuringiensis* strains on *Spodoptera cosmioides*. PJ Neves1, KB Santos1, AM Meneguim1, GT Vilas-Bôas1, WJ Santos1, OM Arantes1, 1Univ Estadual de Londrina-UEL, Londrina-PR, Brazil, 2Instituto Agronômico do Paraná-IAPAR, Brazil

B-26 Study on preparation of *Bacillus thuringiensis* controlling both *Lepidoptera* and *Coleoptera* pests. P Chen1, M Sun2, Z Yu1, S Chen1, H Xie1, G Yu2, 1Zhuai Agricultural Science Research Centre, Zuhai, Guangdong, PR China, 2Dept of Microbial Science and Technology, Huazhong Agricultural Univ, Wuhan, Hubei, PR China

B-27 Molecular dynamics simulation of *Bacillus thuringiensis* Cry4a mosquito-larvicidal protein in explicit water. T Taveecharoenkood1, T Kerdkhaoenroeng1, C Angus thanasomabat1, 1Dept of Immunology, Siriraj Hospital, and 2Dept of Physics and Capability Building Center for Nanoscience and Nanotechnology, Mahidol Univ, Bangkok, Thailand, 3Lab of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol Univ, Nakornpathom, Thailand

B-28 Light and electron microscope investigations on a rickettsial disease of the subterranean burrower bug, *Cytomenes bergi* Froeschner (Hemiptera: Cynidae). RG Kleespies, Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany

B-29 Effects of *Bacillus thuringiensis* on the predatory mite *Euseius concordus* (Acari: Phytoseidae). FH Ibanhes, I Delalibera Jr, Dept of Entomology, Plant Pathology and Agricultural Zoology, ESALO – Univ de Sao Paulo, Brazil

B-30 Impact of *Bacillus thuringiensis* Cry toxins on the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae): *In vivo* binding, histopathological and prey-mediated effects. A Rodrigo-Simón1, C Avila1, JF González-Zamora1, J Ferré1, 1Departamento de Genética, Univ de Valencia, Valencia, Spain, 2Departamento de Ciencias Agroforestales, Univ de Sevilla, Spain
B-33

A common, but complex, mode of resistance of Plutella xylostella to Bacillus thuringiensis toxins Cry1Ab and Cry1Ac. S.Bira-Palacios1, AH Sayyed2, DJ Wright2, N Crickmore1, B Escribe1, 1Dept of Genética, Univ de Valencia, Spain, 2Dept of Entomology, Univ of Sussex, Brighton, East Sussex, UK

B-34

Lack of binding of Bacillus thuringiensis Cry1A toxins as the basis of resistance in a greenhouse-derived population of Trichoplusia ni. A Rodrigo-Simón1, P Wang2, J Zhao2, A Shelton2, J Ferre1, 1Dept of Genética, Univ de Valencia, Spain, 2Dept of Entomology, Cornell Univ, Geneva, NY, USA

B-35

Comparative analysis of Bt toxins binding among susceptible and resistant strains of European corn borer. J González-Cabrera1, HA Siqueira2, BD Siegfried2, J Ferre1, 1Dept of Genética, Facultad de CC. Biológicas, Univ de Valencia, Spain, 2Dept of Entomology, Univ of Nebraska-Lincoln, NE, USA

B-36

Reduction in levels of the Heliotis virescens alkaline phosphatase (HvALP) as a marker for resistance to Cry1Ac. JL Jurat-Fuentes1, MJ Adang1, 1Dept of Entomology and Biochemistry & Molecular Biology, Univ of Georgia, Athens, GA 30602, USA

B-37

Could insect gut esterases be a threat to Bt crops? AH Sayyed1, MJ Bruce2, DJ Wright2, N Crickmore1, 1Dept of Biochemistry, Univ of Sussex, Brighton UK, 2Division of Biology, Imperial College London, Ascot, Berkshire UK

B-38

Analysis of midgut proteinases from Bacillus thuringiensis susceptible and resistant to Heliothis virescens (Lepidoptera: Noctuidae). L Karumbaiah1, B Oppert2, JL Jurat-Fuentes1, MJ Adang1, 1Dept of Entomology, Biochemistry and Molecular Biology, Univ of Georgia, Athens, GA, 2USDA-ARS Grain Marketing and Production Research Center2, Manhattan, KS, USA

B-39

Mixing and matching of toxin complex proteins. T Hey, S Bevan, A Schleper, P Birkhold, S Burton, T Meade, D Merlo, J Sheets, R Thompson, H Moon, Dow AgroSciences, Indianapolis, IN, USA

B-40

Novel toxin complex constructions. T Hey, C Cai, A Woosley, S Burton, J Sheets, B Waldman, H Moon, T Meade, D Merlo, Dow AgroSciences, Indianapolis, IN, USA

B-41

Cloning and expression in a methylotrophic bacterium of an insecticidal crystal protein from Bacillus thuringiensis. L Gringorten1, Y Choi1, L Morel2, L Masson2, C Miguez2, 1Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada, 2Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec Canada
Contributed Papers (Virus Div., Other) Wedn, 8:00-10:00. BEB 117

**Immunity**

**Moderator:** Bruce Webb.

**8:00**

**103** Spodoptera littoralis response to infection with AcMNPV. H Rivkin*, JA Kroemer*, BA Webb*.

**8:15**

**104** Hemocyte variations relating to age related immunocompetency in gypsy moth (Lymantria dispar).

J McNeil, D Cox-Foster, M Grove, K Hoover, Dept of Entomology, Pennsylvania State Univ, University Park, PA, USA.

**8:30**

**105** Molecular cloning of Choristoneura fumiferana prophenoloxidases 1 and 2 and their regulation by a polydnavirus.

D Douse*, Q Feng*, M Cusson*, Laurentian Forestry Centre, Natural Resources Canada, Quebec, Canada, Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, Canada.

**8:45**

**106** Polydnavirus-induced apoptosis of host hemocytes after parasitization of the host lepidopteran Manduca sexta by the parasitoid wasp Cotesia congregata.

RF Dumpt*, NE Beckage*, Dept of Biochemistry and Molecular Biology, and Dept of Entomology & Cell Biology and Neuroscience, Univ of California-Riverside, Riverside, CA, USA.

**9:00**

**107** Genomic analysis of the Drosophila melanogaster innate immune response against a parasitic wasp.

S Albright, D Hultmark, Umeå Centre for Molecular Pathogenesis, Umeå University, Sweden.

**9:15**

**108** Investigating immune functions in mosquito cell lines.

Ann M Fallon, Dept of Entomology, Univ of Minnesota, St. Paul, MN, USA.

**9:30**


**9:45**

**110** Characterization and expression analysis of biodefense-related genes from kuruma shrimp, Marsupenaeus japonicus. TAoki, I Hirose, Lab of Genome Science, Tokyo Univ of Marine Science and Technology, Minato, Japan.
10:45 **124** Bt-horus® SC, a Brazilian bioinsecticide to control mosquitoes and black-flies. R Monnerat¹, CM Soares². ¹Embrapa Recursos Genéticos e Biotecnologia, and ²Bttech Biotecnologia Ltda, Brasilia, Brazil

11:00 **125** Controlled delivery of single and joint-action biolarvicide formulations for control of mosquito larvae. R Levy, MA Nichols, WR Opp, Lee County Mosquito Control District, Technology Development Center, Ft. Myers, FL, USA

11:15 **126** Decreased resistance to Bt cotton in pink bollworm? AJ Gassmann, JA Fabric, MS Sisterson, S Morin, TJ Denney, Y Carrière, BE Tabashnik, Dept of Entomology, Univ of Arizona, Tucson, USA

11:30 **127** Evaluation of two formulations based on microbial metabolites to control blackcurrant insect pests. MV Shternshis¹, MA Vasin¹, VV Gouli². ¹Novosibirsk State Agrarian Univ, Russia, ²Univ of Vermont, USA

11:45 **128** Mortality of gypsy moth (Lymantria dispar) induced by *Bacillus thuringiensis* var. *kurstaki* is inversely related to temperature. K van Frankenhuysen, Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

12:00 **129** Cloning and expression of cry1Ah1 gene from isolate of *Bacillus thuringiensis* and its bioactivity. H Li¹, J Tan¹, L Han², K He¹, G Liang¹, F Song¹, D Huang¹, J Zhang¹. ¹State Key Lab for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Acad of Agricultural Sciences, Beijing, ²College of Life Sciences, Northeast Agric Univ, Harbin, Biotechnology Research Institute, CAAS, Beijing, PR China

12:15 **130** Characterization of a *Bacillus thuringiensis* strain BT185 toxic to the Asian cockchafer: *Holotrichia paralella*. H Yu¹, F Song¹, J Zhang¹, J Gao¹. ¹State Key Lab of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agric Sciences, Beijing, ²Northeast Agricultural Univ, HarBin, PR China

12:30–2:00 **LUNCH** Cuddy Center

**STU**

**WEDNESDAY PM**

12:30 **131** The gestalt of performing: An eclectic guide to successful oral presentations. JD Vandenberg, USDA-ARS, Ithaca, New York, USA

12:42 **132** A good scientific researcher needs to be a good communicator. A Bravo, Instituto de iotechnologia/UNAM, Cuernavaca, Mexico

12:54 **133** The WYSIWYG challenge: The visual aspects of presentation really do matter. R Humber, USDA-ARS, Ithaca, New York, USA

1:06 **134** Where art and precision meet: Presenting data clearly. V D’Amico, Dept of Entomology, Univ of Delaware – USDA-FS, Newark, DE, USA

**Student Workshop and Mixer** Wednesday, 12:30-2:00. BEB 110

**Talking the talk: A “how to” guide.**

**Mixer at 1:20**

Organizer and Moderator: Todd Ugine

13:30 **135** Quantification of the dose of lepidopteran activity in new cotton events expressing the insecticidal protein Vip3A. A D’O Reilly, N Dujon, J Cairns, R Windle, R Hughes, M Gill, A Blake, J Sheridan, Syngenta, Jealotts Hill Research Center, Bracknell, Berks, UK

13:45 **136** Identification of rip3A-type genes from *Bacillus thuringiensis* strains and characterization of two novel *vip3A*-type genes. J Liu¹, F Song¹, J Zhang¹, J Tan¹. ¹State Key Lab of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, ²Agricultural Univ of HeBei, PR China

13:52 **137** Two novel classes of secreted insecticidal proteins of *Bacillus thuringiensis*. WP Donovan¹, JT Engleman², JC Donovan³, WP Clinton¹, OM Ilagan¹, KC Krasomil-Osterfeld¹, TM Malvar¹, JW Pitkin¹, MR Walters¹, JA Baum¹, JK Roberts¹. ¹Monsanto Company, St. Louis, MO, ²Eco-Corporated, Langhome, PA, USA

14:05 **138** Enterotoxigenic genes: Are they involved in insecticidal activity in *Bacillus thuringiensis*? O Kyei-Poku¹, D Gauthier², K van Frankenhuysen, Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

13:00 **139** Cloning and mutation of the cry8C-type gene from *Bacillus thuringiensis* toxic *Anomalol curopalenta*. C Shu¹, F Song¹, S Feng², R Wang², J Zhang¹. ¹State Key Lab of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Beijing, ²Institute of Plant Protection, HeBei Agricultural and Forest Sciences, PR China

13:15 **140** Introduction of *Culex* toxicity to lepidopteran specific *Bacillus thuringiensis* Cry1AA by protein engineering. X Liu, DH Dean, Dept of Biochemistry, Ohio State Univ, Columbus, OH, USA

13:30 **141** The activity of antoxidants in midgut of larvae. JN Dubovsky, EV Grizanova, EA Boiarisheva, VV Glupov, Lab of Insect Pathology, Institute of Animal Systematics and Ecology, Novosibirsk, Russia
V-1 Heterologous baculovirus pathogenicity in the absence of contemporary coevolution. G Moreau1, CJ Lucarotti2, EG Kettela1, KN Barber2, SE Holmes1, SB Holmes2, C Weaver1, R Morin1. 1Natural Resources Canada, Canadian For Serv-Atlantic Forest Centre, Fredericton, New Brunswick, Canada, 2Natural Resources Canada, Canadian For Serv-Great Lakes Forest Centre, Sault Ste. Marie, Ontario, Canada

V-2 Ecosystem alteration modifies the relative strengths of top-down and bottom-up forces in a herbivore population. G Moreau1, E S Eveleigh1, CJ Lucarotti2, DT Quiring3, 1Natural Resources Canada, Canadian For Serv - Atlantic Forest Centre, Fredericton, New Brunswick, Canada, 2Population Ecology Group, Faculty of Forestry and Environmental Management, Univ of New Brunswick, Fredericton, New Brunswick, Canada

V-3 Efficacy of indigenous TsSNPV and AcMNPV isolates for control of Trichopilia ni: Greenhouse cage trials. M Erlandson1, D Gillespie1, M Strom1, D Quiring1, D Theilmann2, 1Natural Resources Canada, Canadian For Serv - Atlantic Forest Centre, Fredericton, New Brunswick, Canada, 2Population Ecology Group, Faculty of Forestry and Environmental Management, Univ of New Brunswick, Fredericton, New Brunswick, Canada

V-4 Relative activity of baculoviruses of the diamondback moth. RR Farrar, Jr., M Shapiro, BM Shepard. 1USDA-ARS, Insect Biocontrol Laboratory, Beltsville, MD, 2Clemson Univ, Charleston, SC, USA

V-5 Aerosol infectivity of baculovirus to insect larvae: A new larval inoculation strategy for baculovirus. T-Y Wu1, T-Y Jinn1, S-S Kao2, JTC Tzeng1, 1, 2Biopesticide Department, Taiwan Agriculture Chemicals and Toxic Substances Research Institute, Wufeng, Taiwan, 3Dept of Biology, National Taiwan Univ, Taipei, Taiwan

V-6 Fall armyworm Spodoptera frugiperda base line of susceptibility to baculovirus SINPV strain from Paraná, Brasil. M Vázquez1, T López2, J Vázquez R. 1Lab of Entomología, División de Ciencias Agronómicas, Univ de Guadalajara, México, 2División de Ciencias Biológicas, Univ de Guadalajara, México

V-7 RAPD-PCR fragments marking resistance and susceptibility of Lymantria dispar to nuclear polyhedrosis virus. AV Iyashov1, AP Simchuk1, VV Oberevkom2, VV Gouli1. 1Dept of Ecology, VI Varnadsky Natl Univ, Ukraine 2Entomology Research Laboratory, Dept of Plant and Soil Science, Burlington, VT USA

V-8 Production of the Lymantria dispar nucleopolyhedrovirus in stirred tank bioreactors. JM Slavicek1, JM Gabler, USDAA Forest Service, Northeastern Research Station, Delaware, OH, USA

V-9 In vitro propagation of NPVs from Lymantria xylina, C-t Ku, C-Y Wu, C-H Wang. Dept of Entomology, National Taiwan Univ, Taipei, Taiwan

V-10 Genetic stability of Erinnys ello granulovirus applied as a bioinsecticide in Brazil. NR Costa1, BC Ferreira2, MEB Castro1, R Pegasoro3, ML Souza3. 1Embrapa Genetic Resources and Biotechnology, Brasdilia-DF, Brazil, 2EPAGRI Santa Catarina S.A., Itajaí-SC, Brazil

V-11 Coral red fluorescence protein as genetic modified baculovirus tracer. T-R Jinn1, 2, S-S Kao2, JTC Tzeng1, T-Y Wu3, 1Graduate Institute of Biotechnology, Natl Chung Hsing Univ, Taichung, Taiwan, 2Biopesticide Department, New Taiwan Agriculture Chemicals and Toxic Substances Research Institute, Wufeng, Taiwan, 3Dept of Bioscience Technology, Chung Yuan Christian Univ, Chung Li, Taiwan

V-12 Short term starvation reduces intrasatal developmental resistance of gypsy moth (Lymantria dispar) to LdNPV. J Harenza1, M Grove1, S Geih3, K Hoover, The Pennsylvania State Univ, Dept of Entomology, PA, USA

V-13 The GP64 protein of AcMNPV rescues HaSNPV transduction in mammalian cells. C Liang1, J Song1, X Chen1, 1State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 2Graduate School of the Chinese Academy of Sciences, Beijing, PR China

V-14 A cellular Drosophila melanogaster protein with similarity to baculovirus envelope fusion proteins. O Lang1, GW Blissard1, 1Dept of Biological Sciences, Univ of Lethbridge, Canada, 2Boyce Thompson Institute, Cornell Univ, Ithaca, NY, USA

V-15 Analysis of the CDMPV IAP genes. J de Jong1, BM Ariës1, BA Thelmann2, PJ Krell1, 1Dept of Molecular and Cellular Biology, Univ of Guelph, ON, Canada, 2Great Lakes Forest Centre, Canadian Forest Service, Sault Ste. Marie, ON, Canada, 3Agoura and Agri-Food Canada, Summerland BC, Canada

V-16 Screening of cellular factors which interact with Host Range Factor-1 (HRF-1) from Lymantria dispar nucleopolyhedrovirus. H Ishikawa1, M Ikeda1, SM Tchium2, M Kobayashi1, 1Graduate School of Bioagricultural Sciences, Nagoya Univ, Chikusa, Nagoya, Japan, 2Dept of Entomology and Microbiology and Molecular Genetics, Michigan State Univ, East Lansing, MI, USA

V-17 Characterization of the gp41 gene of Spodoptera litura multicaispis nucleopolyhedrovirus. L Pan, Z Li, Y Gong, M Yu, K Yang, Y Fang, State Key Laboratory of Biocontrol, Zhongshan Univ, Guangzhou, PR China

V-18 Molecular cloning and functional characterization of a putative glycosyltransferase family 8 member Lsp13 in Leucania separata multiple nuclear polyhedrosis virus. Y Liu1, E Du, H Xiao, W Jin, Y Qi, State Key Lab of Virology, College of Life Science, Wuhan Univ, Wuhan, PR China

V-19 Functional analysis of FP25K of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus. D Wu1, F Deng1, X Sun1, H Wang1, L Yuan1, JML Vlak1, Z Hu1, State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China, 2Lab of Virology, Wageningen Univ, Wageningen, The Netherlands

V-20 Characterization of an AcMNPV without virions occluded in the polyhedra. L Wang, X-W Cheng, Dept of Microbiology, Miami Univ, Oxford, Ohio, USA

V-21 Analysis of the temporal expression of Trichoplusia ni single nucleocapsid nucleopolyhedrovirus genes following transfection of Ta5-B1 cells. M van Munster1, L Willis1, M Elias1, M Erlandson1, D Thelmann2, R Brousseau1, L Masson1.


**Microsporidia and Protozoa**

**MP-1** The Eppendorf® - micromanipulator - a new technique for the quantitative separation of microsporidian spores for infection experiments. T Kolling, D Pilarska, A Linde.

**MP-2** A microsporidium infecting the black vine weevil, *Otiorychus salcatus* (F) (Coleoptera: Curculionidae). DJ Brick, L. Solter.

**MP-3** *Nosema ceranae* infection in *Apis mellifera*. W-F Huang, J-H Jiang, C-H Wang, Dept of Entomology, National Taiwan Univ, Taiwan.

**MP-4** Phylogenetic analysis of the *Nosema* spp. from cruciferous lepidopteran pests in Taiwan. K Chin-Tai, C-C Tseng, C-Ch Wang, Dept of Entomology, National Taiwan Univ, Taipei, Taiwan.


**Microbial Control**

**MC-1** Intraguild interactions between *Verticillium lecanii* (Zimmermann) Viegas and *Aphidolates aphidomyza* (Diptera: Cecidomyiidae) as biological control agents of *Myzus persicae* (Homoptera: Aphididae). P Jaramillo, B Roitberg, M Goettel, D Gillespie.


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**WEDNESDAY PM**

V-22 Organization of the *Choristoneura occidentalis* granulovirus genome. SR Coppons, HAM Lauzon, PJ Kreli, BM Arif. Great Lakes Forestry Centre, Sault Ste. Marie ON, Dept of Microbiology, Univ of Guelph, ON, Canada.


V-24 The genome sequence of *Chysoselitix chalcites* nucleopolyhedrovirus, a baculovirus with two DNA photolyase genes. MM van Oers, MHC Abma-Henkes, EA Henning, JML Vlak. 1Lab of Virology, Wageningen Univ, The Netherlands, 2Greenomics, Plant Research International BV, Wageningen, The Netherlands, 3Dept of Biological Sciences, Imperial College London, UK.


V-27 In vivo cloning and comparative characterization of eleven distinct entomopoxviruses isolated from sympatric populations of *Adoxophyes honmai* and *Homona maganinam* (Lepidoptera: Tortricidae). T Ishii, Y Takahashi, J Takatsuka, S Okuno, K Nakinishi, M Nakai, Y Kunimi. Dept of Bioregulation and Biointeraction, Tokyo Univ of Agriculture and Technology, Fuchu, Tokyo, Japan.

V-28 Searching for a homologue of the *Mythimna separata* entomopoxvirus gene encoding the protein lethal to the endoparasitoid *Cotesia kariyai*. E Izuka, S Okuno, M Nakai, T Ishii, J Takatsuka, K Nakinishi, Y Kunimi, Dept of Bioregulation and Biointeraction, Tokyo Univ of Agriculture and Technology, Fuchu, Tokyo, Japan.

V-29 Promoter analysis of *Cotesia plutellae* polydnavirus and application for improved insecticides. JY Choi, JN Kang, YS Kim, Y Wang, HK Choi, MS Li, YH Je, School of Agricultural Biotechnology, Seoul National Univ, Korea.

V-30 Changed to oral presentation

V-31 Histopathological diagnosis of *Perina nuda* (Lepidoptera: Lynantidae) infected with PnPV (*Perina nuda* picorna-like virus). C-Y Wu, C-H Wang, Dept of Entomology, National Taiwan Univ, Taipei, Taiwan.

V-32 Modulation of GAPDH and fructose-biphosphate metabolism in shrimps after white spot syndrome virus (WSSV) infection. H-C Wang, H-C Wang, S-E Peng, C-F Lo, S-H Chiu, 1Institute of Biochemical Sciences, and 2Institute of Zoology, National Taiwan Univ, Taipei, Taiwan.

V-33 Identification and application of P9, the most highly expressed gene of WSSV. H-C Wang, C-F Lo, G-H Kou, Institute of Zoology, National Taiwan Univ, Taipei, Taiwan.

V-34 Identification of the nucleocapsid, tegument and envelope proteins of the shrimp white spot syndrome virus virion. J-M Tsai, H-C Wang, G-H Kou, C-F Lo, Institute of Zoology, National Taiwan Univ, Taipei, Taiwan.

V-35 Identification of basal promoter and enhancer regions in an untranslated region of WSSV iew. W-J Liu, C-F Lo, G-H Kou, Institute of Zoology, National Taiwan Univ, Taipei, Taiwan.

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**STU** aldolase expression in shrimps after white spot syndrome virus (WSSV) infection. H-C Wang, H-C Wang, S-E Peng, C-F Lo, S-H Chiu, 1Institute of Biochemical Sciences, and 2Institute of Zoology, National Taiwan Univ, Taipei, Taiwan.
MC-3 Factors that influence the desiccation tolerance and storage stability of blastospores of the entomopathogenic fungus <i>Paecilomyces fumosoroseus</i>. MA Jackson1, S Cliquet2, S Erhan3, WJ Connick, Jr.1 USDA-ARS, Natl Center for Agricultural Utilization Research, Peoria, IL, USA, 2Lab Univ de Microbiologie Appliquée de Quimper, France, 3Georgia-Pacific Corporation, IL, USA, 4USDA-ARS, New Orleans, LA, USA

MC-4 Considerations in using <i>Metarhizium anisopliae</i> as a biopesticide for wireworms. JT Kabaluk1, MS Goettel2, RS Vernon1. Agriculture and Agri-Food Canada - Pacific Agri-Food Research Centre, Agassiz, BC, 1Lethbridge Research Centre, Alberta, Canada

MC-5 Virulence of fungal biocontrol agent <i>Beauveria bassiana</i> to the eggs and adults of carmine spider mite <i>Tetranychus cinnabarinus</i>. W-B Shi1, M-G Feng1, 2, Institute of Applied Entomology, Coll of Agriculture and Biotechnology, Zhejiang Univ, PR China, 2Institute of Microbiology, College of Life Sciences, Zhejiang Univ, PR China

MC-6 Development of <i>Beauveria bassiana</i>-based mycoinsecticide for tea leafhopper control in China: Current status and prospects. M-G Feng1, 2, S-H Ying1, 1Institute of Microbiology, College of Life Sciences, Zhejiang Univ, PR China, 2Institute of Applied Entomology, Coll of Agriculture and Biotechnology, Zhejiang Univ, PR China

MC-7 Modeling analysis of the interaction of <i>Beauveria bassiana</i> and imidacloprid on two aphid pests. S-D Ye1, M-G Feng1, 2, 1Institute of Microbiology, College of Life Sciences, Zhejiang Univ, PR China, 2Institute of Applied Entomology, Coll of Agriculture and Biotechnology, Zhejiang Univ, PR China

MC-8 Quantified interaction of fungal biocontrol agent <i>Beauveria bassiana</i> and a thiosulphate-diaminom nitrocellulose on <i>Plutella xylostella</i> larvae. L Tian1, M-G Feng1, 2, 1Institute of Microbiology, College of Life Sciences, Zhejiang Univ, PR China

MC-9 Susceptibility of larval stages of the aphid parasitoids <i>Aphidius colemani</i> and <i>A. matricariae</i> inoculated with the entomopathogenic fungus <i>Beauveria bassiana</i>. M Filotas1, J Sanderson1, S Wright1. 1Dept of Entomology, Cornell Univ, and 2USDA-ARS, US Plant, Soil, & Nutrition Laboratory, Ithaca, NY, USA

MC-10 Compatibility and potential synergism between the entomopathogenic fungus <i>Beauveria bassiana</i> and the insect growth regulator azadirachtin for control of the greenhouse pests <i>Mycus persicae</i> and <i>Aphis gossypii</i>. M Filotas1, J Sanderson1, S Wright1. 1Dept of Entomology, Cornell Univ, and 2USDA-ARS, US Plant, Soil, & Nutrition Laboratory, Ithaca, NY, USA

MC-11 Toxicity analysis of truncated insecticidal crystal proteins Cry1Ba3 from <i>Bacillus thuringiensis</i>. G Wang1, J Zhang1, J Wu1, F Song1, D Huang2, 1State Key Lab for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, and 2Institute of Biotechnology Research, Chinese Academy of Agricultural Sciences, Beijing, PR China

MC-12 Is phenoloxidase involved in induced resistance to <i>Bacillus thuringiensis kurstaki</i> in <i>Trichoplusia ni</i>? AF Jammant1, J Manson2, 1, J Ericson1, V Caron1, JH Myers1. 1Dept of Biological Sciences, Simon Fraser Univ, Burnaby, BC, Canada, 2Dept of Zoology, Univ of Toronto, Ontario, Canada, 3Dept of Zoology, Univ of British Columbia, Vancouver, BC, Canada

MC-13 Construction of a <i>Bacillus thuringiensis</i> BAC library and partial cloning of zwittermicin A biosynthesis cluster. T Shao1, F Song1, J Zhang1, D Liu1, D Huang1, 1State Key Lab of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, and 2Institute of Biotechnology Research, Chinese Academy of Agricultural Sciences, Beijing, 3Agricultural Univ of HeHei, BaoDing, PR China

MC-14 Implementation of the largest worldwide laboratory production of a baculovirus: The case of the nucleopolyhedrovirus of <i>Anticarsia gemmatalis</i> (Lep.: <i>Noctuidae</i>) in Brazil. F Moscardi1, B Santos2, 1Embrapa Soja, Londrina, PR, Brazil, 2Univ Federal do Parana, Curitiba, PR, Brazil

MC-15 Laboratory and orchard studies on the transmission of <i>Cydia pomonella granulovirus</i> by contaminated <i>C. pomonella</i> adults. D Winstanley1, JV Cross2, N Naish1, G Keane1, S Hilton1, DJ Gajek2, R van Wezel2, 1Warwick HRI, Wellesbourne, Warwick UK, 2East Malling Research, West Malling, Kent, UK

NEMATODES

N-1 Evaluation of a native <i>Heterorhabditis</i> species from the Coastal Region of Central Peru against white grubs. J Alcazar1, C Farfán2, J Salazar3, C Castillo2, HK Kaya1, 1International Potato Center, Lima, Perú, 2Institute Valle Grande, Cañete, Perú, 3Dept of Nematology, Univ of California, Davis, CA, USA

N-2 Targeting the Andean weevils with a native entomopathogenic nematode species. S Parsa1, J Alcazar2, L Lizarra3, HK Kaya4. 1Dept of Nematology, Univ of California, Davis, CA, USA, 2International Potato Center, Lima, Peru, 3CRIBA, Univ of Cusco, Cusco, Peru

N-3 Virulence of various commercial isolates of <i>Heterorhabditis bacteriophora</i> against the European chafer (Rhizotrogus majalis). L Simard2, D Winstanley1, JV Cross1, 1Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, Quebec, Canada, 2Royal Canadian Golf Association, Oakville, Ontario, Canada

N-4 Entomopathogenic nematode production enhancement using physical and chemical host stressors. IM Brown1, DI Shapiro-Ilan1, R Gaugler1, 1Biology, Georgia Southwestern State Univ, Americus, GA, 2USDA-ARS, SE Fruit and Tree Nut Research Lab, Byron, GA, 3Entomology, Rutgers Univ, New Brunswick, NJ, USA

N-5 Infectivity of entomopathogenic nematodes and immune responses of their insect hosts. X Li1, J Alcázar2, 1Warwick HRI, Wellesbourne, Warwick UK, 2Entomology, The Pennsylvania State Univ, PA, 3The Connecticut Agricultural Experiment Station, Windsor, CT, 4Dept of Biology, Eastern Connecticut State Univ, Willimantic, Connecticut, 5Dept of Entomology, Rutgers Univ, New Brunswick, NJ, USA

OTHER

O-1 The <i>Sleeping Beauty</i> transformation system: A new approach for the study of tick cell microbe interactions. TJ Kurtti1, RF Felsheim1, GD Baldridge1, NY Burkhardt1, MJ Herron1, UG Munderloh1. 1Dept of Entomology, Univ of Minnesota, St. Paul, MN, USA
Symposium (Cross-Div.)  Wednesday, 4:30–6:30. BEB 110

Molecular interactions between insect vectors and human pathogens
Organizer/Moderator: Liwang Cui.

4:30  143 Molecular interactions between the malaria parasite and its mosquito vector. M Jacobs-Lorenz, Johns Hopkins Univ, USA

5:00  144 Relationships between the symbiont Sodalis glossinidius and the vectorial competence of tsetse flies. A Geiger, G Cuny, R Frutos, Campus International de Baillarguet, Montpellier, France

5:30  145 Functional genomics in the postgenomic era: What do we learn from the apicomplexan malaria parasite? L Cui, Pennsylvania State Univ, USA

6:00  146 Sand fly midgut receptors for Leishmania parasites: Targets for transmission-blocking vaccines. J Valenzuela, NIAID, National Institutes of Health, USA

Contributed Papers  Wednesday, 4:30–6:30. BEB 117

MICROBIAL CONTROL 2
Moderator: Lawrence Lacey.

4:30  147 Interactions between the granulovirus PoGV and Bacillus thuringiensis (Berliner) against the potato tuber moth, Phthorimaea operculella (Zeller). M Sporleder1, D Mamani2, J Huber1, J Kroschel1. 1International Potato Center, Lima, Peru, 2Univ Nacional Mayor de San Marcos, Lima, Peru, 3Institut of Biological Control, Federal Biological Research Center for Agriculture and Forestry, Darmstadt, Germany

4:45  148 Optimizing the use of the codling moth granulovirus: Effects of application rate and frequency of spraying on control of codling moth larvae in Pacific Northwest apple orchards. LA Lacey1, S Arthurs1, HL Headrick1, R Fritts, Jr1. 1USDA-ARS, Yakima Agricultural Research Lab, Wapato, WA, 2Certis USA, Clovis, CA, USA

5:00  149 Semiochemical driven auto-dissemination of viruses for the control of orchard pests. D Winstanley1, JV Cross2, N Naish1, G Keane1, S Hilton1. 1Warwick HRI, Univ of Warwick, Wellesbourne, UK, 2East Malling Research, West Malling, Kent, UK

5:15  150 Biotic and abiotic factors affecting the field persistence and residual efficacy of Cryptoplea leucotreta granulovirus on citrus. SD Moore, W Kirkman, Citrus Research International, Humewood, Port Elizabeth, South Africa

5:30  151 Can pathogens be used for eradication of soil pests? TA Jackson, T Kleinschaefer, RJ Townsend, AgResearch, Lincoln, New Zealand

5:45  152 Efficacy of entomopathogenic nematodes, applied in an insect cadaver, as biological control agent against soil-dwelling stages of bollworm (Helicoverpa armigera Hübner). A Jankielsohn, JL Hatting, ARC-Small Grain Institute, Bethlehem, South Africa

6:00  153 Combined use of insect pathogenic fungi and nematodes against the onion thrips, Thrips tabaci in the field. K Jang, Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany

6:15  154 Construction of the rfp gene marker system to monitor insecticidal and anti-fungal engineered bacterium of Pseudomonas fluorescens Biop8. Y Jia1,2; J Zhang1, G Li1. 1State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 2Agricultural Univ of Hebei, Baoding, and 3Agricultural College of LaiYang, PR China

Contributed Papers  Wednesday, 4:30–6:30. BEB 117

STU

4:30  155 Specific binding of AcMNPV ODV to midgut cellular targets is mediated by pf6 genes Ac022 and Ac119, but not Ac115. JO Washburn, EW Sid, R Sitapara, T Ohkawa, LE Volkman, Dept of Plant and Microbial Biology, Univ of California, Berkeley, CA, USA

4:45  156 Impact of the peritrophic matrix on baculoviral pathogenesis in a tritrophic system. RC Pymalak, K Hoover, Dept of Entomology, Pennsylvania State Univ, University Park, PA, USA

5:00  157 The effect of tannic acid on gypsy moth performance and susceptibility to the nuclear polyhedrosis virus. VV Martemyanov, ZO Markina, SA Romancev, SA Bahvalov, Lab of Insect Pathology, Institute of Animal Systematics and Ecology, Novosibirsk, Russia

5:15  158 Stimulation of cell motility by a viral fibroblast growth factor homolog: Proposal for a role in viral pathogenesis. C Detvisitsakun, MF Berretta, C Lehiy, AL Passarelli, Division of Biology, Molecular, Cellular, and Developmental Biology Program, Kansas State Univ, Manhattan, KS, USA

5:30  159 Pathology of NeahNPV-infection in Balsam Fir Sawfly, Neodiprion abietis larvae. D Whittome1, B Morin2, C Lucarotti2, D Quiring3, D Levin1. 1Univ of Victoria, Victoria, BC, Canada, 2Natural Resources Canada, Canadian Forestry Service, and 3Faculty of Forestry and Environmental Management, Univ of New Brunswick, Fredericton, NB, Canada

5:45  160 Characterization of Helicoverpa armigera nucleopolyhedrovirus ORF2. Y Nie1,2, Q Wang1,3, C Liang1,2, Z Yu1, X Chen1. 1State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Virology, Wuhan, 2Institution of Entomology, Central China Normal Univ, Wuhan, 3Graduate School of the Chinese Academy of Sciences, Beijing, PR China

6:00  161 Co-opting actin and the Arp2/3 complex for baculovirus progeny production. E Goley1, T Ohkawa2, M Welsh1, L Volkman1,2. 1Dept of Molecular and Cell Biology, and 2Dept of Plant and Microbial Biology, Univ of California, Berkeley, CA, USA

6:15  162 Analysis of the ability of exon0 homologues from heterologous baculoviruses to complement an AcMNPV exon0 (orf141) knockout mutant for the production of budded virus. X Dai1,2, BM Ariff, PJ Krell1. 1DA Thelma, Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada, 2Dept of Molecular and Cellular Biology, Univ of Guelph, Ontario, Canada, 3Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste Marie, Ontario, Canada

DINNER  6:30–8:00: Creekside Eatery – “dinner to go” recommended for business meeting attendees
8:30 163 Methods for analysis of data from bioassays with insect pathogens. J Thong, USDA-ARS, Manhatten, Kansas, USA

Additional featured speakers:
J Vandenberg, USDA-ARS, Ithaca, New York, USA
M Goettel, Lethbridge Research Centre, Alberta, Canada

8:30 164 Some effects of compaction on microsporidian nuclear genomes. B Williams, P Keeling, Dept of Botany, Univ of British Columbia, Vancouver, Canada

8:30 165 Microsporidian parasites of crustacea, specificity, sex and populations. J Smith, School of Biology, Leeds Univ, Leeds, UK

9:00 166 Epizootiology of Thelohania solenopsae in the red imported fire ant, with emphasis on social form of the host. J Fuxa, Dept of Entomology, Louisiana State Univ, AgCenter, Baton Rouge, USA

9:30 167 The genus Brachioda and human skeletal muscle infection caused by the mosquito microsporidium, B. algerae. A Calvi, Dept of Biological Sciences, Rutgers Univ, Newark, NJ, USA

Contributed Papers – Thursday, 8:00-10:00. BEB 110

VIRUSES 4
Moderator: Lorena Passarelli.

8:00 168 Establishment of a natural phylogeny of Baculoviruses. R Haushchild1, M Lange1, O Bininda-Emonds2, JA Jehle1. 1Labor for Biotechnologischen Pflanzenschutz, Dienstleistungszentrum Ländlicher Raum Rheinpfalz, Breitenweg, Germany, 2Lehrstuhl für Tierzucht, Technische Univ München, Freising-Weihenstephan, Germany

8:15 169 Whole genome sequence analysis of a Polish isolate of Agrotsis segetum nucleopolyhedrovirus. AK Jakubowska1,2, RMK Lankhors1, J Ziemnicka1, JM Vlak1, 1Lab of Virology, Wageningen Univ, and 2Greenomics, Plant Research International, Wageningen, The Netherlands

8:30 170 Genome sequence and organization of the Neodiprion abietis nucleopolyhedrovirus. S Duffy1, B Morin1, C Lucaroni1, D Levin2. 1Univ of Victoria, Victoria, BC, Canada, 2Natural Resources Canada, Canadian Forestry Service, Fredrickton NB, Canada

8:45 171 Morphological, molecular, and genomic characterization of two mosquito Cypoviruses. TB Green1, A Sharpio1, S White1, S Rao2, P Mertens2, G Carner2, JJ Becnel3, 1USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, 2Pirbright Lab, Institute for Animal Health, Woking, Surrey, UK, 3Clemson Univ, Clemson, SC, USA

9:00 172 Integration of an ichnovirus genome segment in the genomic DNA of lepidopteran cells. D Doucet1, A Levasseur1, C Béliveau1, D Stoltz2, M Cusson1. 1Laurentian Forestry Centre, Sainte-Foy, QC, Canada, 2Dept of Microbiology and Immunology, Dalhousie Univ, Halifax, NS, Canada

9:15 173 Comparison of genome organization and encoded proteins in campoplegine and banchine ichnoviruses. R Lapointe1, BA Webb2, K Tanaka1, W Barney2, D Stoltz3, M Cusson1. 1Laurentian Forestry Centre, Sainte-Foy, QC, Canada, 2Dept of Entomology, Univ of Kentucky, Lexington, USA, 3Dept of Microbiology, Dalhousie Univ, Halifax, Canada

9:30 174 Display of a foreign protein using recombinant baculovirus occlusion bodies: A novel vaccination tool. R Wilson1, Y Je1, L Bugeon1, U Straschil1, DR O’Reilly1, IA Olszewska1, 1Dept of Biological Sciences, Imperial College London, UK, 2Biology Dept, Shippensburg Univ, PA, USA

9:45 175 flashBAC: A baculovirus expression system for automated, high throughput production of proteins. L King1, K Richards2, R Hitchman1, H Irving2, S Mann1, E Siaterli1, R Possee1, 1School of Biological and Molecular Sciences, Oxford Brookes Univ, UK, 2NERC CEH, Oxford, UK

Contributed Papers – Thursday, 8:00-10:00. BEB 110

Fungi 3
Moderator: Richard Humber and Ann Hajek.

8:00 176 Field trials of Beauveria bassiana GHA for control of the emerald ash borer. H Liu1, LS Bauer1,2, 1Dept of Entomology, Michigan State Univ, and 2USDA Forest Service, North Central Research Station, E. Lansing, MI, USA

8:15 177 A proactive approach to the use of fungal biopesticides to manage sucking insects in pulp crops in Australia. K Knight1,2, C Hauxwell1,2, C Holdom3, G Simpson1, 1DPI&F Biopesticides Unit, Indooroopilly, 2School of Integrative Biology, Univ of Queensland, St Lucia, Australia, 3DPI&F, Toowoomba, Queensland, Australia

8:30 178 Evaluation of some hypomyzoceteous fungi for the control of glassy-winged sharpshooter, Homalodisca coagulata (Homoptera: Cicadellidae). SK Dara1, MR McGuire2, HK Kay1. 1Shafter Research and Extension Center CA, 2USDA-ARS, Shafter, CA, 3Dept of Nematology, Univ of California, Davis, CA, USA

8:45 179 Field testing of selected Beauveria bassiana isolates against Lygus hesperus in California. MR McGuire1, JE Leland1, 1USDA-ARS, Shafter, CA, USA, 2USDA-ARS, Stoneville, MS, USA
THURSDAY AM

9:00 180 Selection and field evaluation of Beauveria bassiana isolates for control of tarnished plant bug, Lygus lineolaris. JF Leland1, MR McGuire1, J Gont1. USDA-ARS, SIMRU, Stoneville, MS, 2USDA-ARS, SREC, Shafter, CA, USA

9:15 181 Fungal BCAs: Potential control agents to control subterranean pests. H Strenger2, B Pernius1, RK Morelli2. 1Institute of Microbiology, Leopold Franzens Univ Innsbruck, Austria, 2Agrifitur Srl, Brescia, Italy

9:30 182 To germinate or not? Strategies of Beauveria bassiana for survival in soil. CV Manders1, TA Jackson2. B Chapman1. 1Bio-Protection and Ecology Division, Lincoln Univ, and 2AgResearch, Lincoln, Canterbury, New Zealand

9:45 183 Metarhizium anisopliae conidia produced under environmental and nutritional stresses exhibit increased virulence and tolerance to UV-B radiation and heat. DEN Rangel, AJ Anderson, DW Roberts, Dept of Biology, Utah State Univ, Logan, UT, USA

10:00–10:30 BREAK BEB Lobby

THURSDAY PM

10:45 191 Differential expression of cry toxin in a Bacillus thuringiensis strain with dual insecticidal activity. J Torres1, NA Valdez-Cruz1, JD Tinoco2, S Orduz3. 1Unidad de Biotecnología y Control Biológico, Corporación para Investigaciones Biológicas, Carrera, Colombia, 2Coltabaco, Compañía Colombiana de Tabaco S.A., Colombia, 3Univ de Pamplona, Pamplona, Santander, Colombia

1:00–1:30 LUNCH Cuddy Center

IMPORTANT NOTE: Remove all posters before 1:00 pm!

Symposium (Division of Bacteria) Thursday, 1:30–3:30. BEB 101

Toxin-receptor interactions and mode of action

Organizers/Moderators: Mario Soberón and Jeroen van Rie.

1:30 192 Influence of the physico-chemical and biochemical environment on the kinetics of pore formation by Cry toxins. V Vachon, Univ of Montreal, Quebec, Canada

2:00 193 Comparisons of Bt receptors and applications for pest insect control. M Adang, Dept of Biochemistry and Molecular Biology, Univ of Georgia, Athens, USA

2:30 194 Structural and functional analysis of the pre-pore and membrane inserted pore of Cry1A toxins. A Bravo, Instituto de Biotecnología, Univ Nacional Autonoma de Mexico, Morelos, Mexico

3:00 195 Toxin binding site of the Heliothis virescens cadherin. S Gill, Dept of Cell Biology and Neuroscience, Univ of California, Riverside, USA

Symposium (Division of Nematodes) Thursday, 1:30–3:30. BEB 111

Ecology of entomopathogenic nematodes

Organizer/Moderator: Panwinder Grewal.

1:30 196 Biogeographic distribution and diversity of entomopathogenic nematodes: Natural patterns or human-biased trends? P Stock, Univ of Arizona, USA

1:45 197 Relating entomopathogenic nematode presence and abundance to habitat variation in an agroecosystem. C Hoy, Ohio State Univ, Wooster, Ohio, USA

2:00 198 Host-finding and infection decisions in the soil. E Lewis, Univ of California, Davis, USA

2:15 199 Formulations and methods for enhancing post-application survival. D Shapiro-Ilan, USDA-ARS, USA

2:30 200 Abiotic factors affecting success of entomopathogenic nematodes in the field. L Lacey, USDA-ARS, USA
2:45  **201** Biotic factors and farming systems affecting persistence and recycling. M Barbercheck, R Jabbour, Pennsylvania State Univ, USA

3:00  **202** Recycling and long-term persistence of entomopathogenic nematodes. A Koppenhofer, Rutgers Univ, New Brunswick, New Jersey, USA

3:15  **203** Ecology of entomopathogenic nematodes: Past, present and future. H Kaya, Univ of California, Davis, USA

**Symposium (Div. of Viruses)**

**Insect expression systems, gene therapy and vaccine development**

Organizers: James Slavicek and Bryony Bonning.
Moderator: James Slavicek.

4:00  **212** Protein N-glycosylation in the baculovirus-insect cell system. DL Jarvis, JJ Aumiller, JR Hollister, RL Harrison, Dept of Molecular Biology, Univ of Wyoming, Laramie, Wyoming, USA

4:30  **213** Densovirus-derived vectors for stable expression of foreign proteins in insect cells and somatic transformation of insects. M Bergeon, Lab de Pathologie Comparee, Univ Montpellier II, Montpellier, France

5:30  **215** Tailoring the baculovirus insect cell expression system for the production of subunit vaccines. M van Oers, Lab of Virology, Wageningen Univ, The Netherlands

**BACTERIA 4**

Moderator: Colin Berry.

4:00  **216** New *Bacillus sphaericus* toxin genes in strains able to overcome binary toxin resistance in *Culex* larvae. C Berry, GW Jones, Cardiff School of Biosciences, Cardiff Univ, UK

4:15  **217** Toxicity and synergy of Mtx-1 and Mtx-2 toxins from *Bacillus sphaericus* against susceptible and resistant lines of *Culex quinquefasciatus*. MC Wirth, C Berry, Y Yang, WE Walton, BA Federici. Dept of Entomology, Univ of California, Riverside, CA, USA, ‘Cardiff School of Biosciences, Cardiff Univ, Wales, UK

4:30  **218** Pore-forming determinants of *Bacillus thuringiensis* Cry4 mosquito-larvicidal proteins. C Angsusathanasombat, Lab of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol Univ, Thailand
<table>
<thead>
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<th>Time</th>
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<td>4:30</td>
<td>Microbial control of the banana weevil, <em>Cosmopolites sordidus</em>, with <em>Beauveria bassiana</em>. CS Gold¹, C Nankinga¹², W Tinzaara¹, T Dubois¹, J Akello¹, W Tushemereirwe². ¹Intl Institute of Tropical Agriculture, Southern and Eastern Africa Regional Centre, Namulonge, and ²Uganda National Banana research Programme, Kampala, Uganda</td>
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<td>4:45</td>
<td>Effects of day versus evening application times on efficacy of <em>Beauveria bassiana</em> foliar sprays against Colorado potato beetle. S Wraight, ME Ramos, USDA-ARS-PPRU, US Plant, Soil, and Nutrition Lab, Ithaca, New York, USA</td>
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<td>5:00</td>
<td>Use of genetic diversity in <em>Beauveria bassiana</em> for improving the biological control of the coffee berry borer. LP Cruz¹, AL Gaitan², CE Gongora¹. ¹Dept of Entomology, and ²Dept of Plant Pathology, Natl Centre of Coffee Research, Chinchina, Caldas, Colombia</td>
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<td>5:15</td>
<td>The application of <em>Metarhizium anisopliae</em> and <em>Beauveria bassiana</em> for the control of the longicorn beetle borer <em>Agrianome spinicollis</em> (Cerambycidae) in pecan trees. IR Newton¹, A Ward². ¹Stahmann Farms, Trawalla, Pallamallawa, NSW, Australia, ²Becker Underwood, Somersby, NSW, Australia</td>
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<td>5:30</td>
<td>Microbial control of the spotted stemborer <em>Chilo partellus</em> with <em>Beauveria bassiana</em> and <em>Metarhizium anisopliae</em> from Ethiopia and South Africa. T Teferra. Alemaya Univ, Dept of Plant Sciences, Ethiopia</td>
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7:00p-1:00a  BANQUET & Captain Cook Hotel AWARDS CEREMONY

6:30p-7:15p  Buses from UAA housing
7:00p-8:00p  Cocktail hour
8:00p  Banquet
10:00p-2:00a  Buses back to UAA housing

~ Thanks for coming to Anchorage! ~

We hope to see you in 2006 in Wuhan!
IMPORTANT NOTES:
These abstracts should not be considered to be publications and should not be cited in print without the author’s permission.

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

129 indicates abstract number for ORAL presentation

B-11 indicates abstract number for POSTER presentation
Overall, most differences in gene expression involved perception soildwelling and insect pathogenic life-stages. As *M. anisopliae* fungal pathogens currently testing. previously unsuspected stratagems of fungal pathogenicity that we are employing cDNA microarray analyses that identify different subsets several different host environments. We shall present studies duplication/loss, and gene divergence. In addition, like many other where strains with broad host ranges and strains with very narrow model to study the role of gene duplication/divergence in generating for virulence in pathogens. The tremendous amount of genetic Host pathogen interactions are an important force shaping organismal impact. Invertebrate pathogens: Evolution and impact

Symposium. Monday, 10:30.

One step ahead of emerging crustacean viruses

Chu-Fang Lu and Guang-Hsiung Kou

Institute of Zoology, National Taiwan University, Taipei, Taiwan

The last twenty years have seen the emergence of several new crustacean viruses. Some of these have had relatively little impact, while others, such as white spot syndrome virus (WSSV) and Taura syndrome virus, grew very quickly into global epidemics with high mortality and serious economic losses. This review will focus on WSSV, which is the causative agent of white spot disease (WSD). WSSV is a large (~300 kbp) dsDNA virus that first appeared in 1992 in Fu-Jian province, China. Initially classified as a baculovirus, in 2002 WSSV was erected as the type species of a new genus, *Whisphovirus*, in the new family Nimaviridae. The uniqueness of WSSV means that other viral infection models often cannot be applied, and the functional genomics of the virus need to be studied *ab initio*. Nevertheless, since its devastating emergence, the development of reliable, easy-to-use diagnostic tools and our increased knowledge of the disease and the virus have led to improved culturing and monitoring techniques and enabled other antiviral measures such as stricter quarantine controls. Strategies that are currently being explored include the development of disease resistant strains, shrimp lines that are specific pathogen free, and gene targeting. Recent vaccination studies have also suggested that the crustacean defense system may possess pathogen-specific memory.

Symposium. Monday, 11:00.

Molecular adaptations for pathogenicity in *Metarhizium anisopliae*

Raymond J. St. Leger

Department of Entomology, University of Maryland, College Park, MD 20742, USA

Host pathogen interactions are an important force shaping organismal diversity, yet little is known about the evolution of genes responsible for virulence in pathogens. The tremendous amount of genetic variation, distinct disease phenotypes and host ranges of strains of the insect pathogen *Metarhizium anisopliae* have made it an excellent model to study the role of gene duplication/divergence in generating the functional diversification of enzymes and toxins necessary for adaptation to different hosts. To illustrate this, we present examples where strains with broad host ranges and strains with very narrow host ranges have diverged through changes in gene regulation, gene duplication/loss, and gene divergence. In addition, like many other fungal pathogens *M. anisopliae* is a facultative saprophyte with both soil-dwelling and insect pathogenic life-stages. As *M. anisopliae* traverses the cuticle and enters the hemolymph it must also adapt to several different host environments. We shall present studies employing cDNA microarray analyses that identify different subsets of genes allowing physiological adaptation to insect cuticle, insect hemolymph and bean root exudate (a model for life in the soil). Overall, most differences in gene expression involved perception mechanisms, carbon metabolism, proteolysis, cell surface properties and synthesis of toxic metabolites. These differences suggest many previously unsuspected stratagems of fungal pathogenicity that we are currently testing.

Symposium. Monday, 12:00.

Invertebrates as a source of emerging human pathogens

R. Ffrench-Constant and N. Waterfield

Biology, University of Bath, Bath BA2 7AY, UK

Despite their importance, little is known about the origins of emerging human pathogens. We argue that given the age and predominance of bacteria-invertebrate interactions, that these pathogens have 'learned' their pathogenic skills in invertebrates rather than vertebrates. Thus the current emphasis on sequencing pathogens of man is only giving us a tiny glimpse of the available net 'pathosphere' (the global pool of virulence factors). We will discuss the communalities of the vertebrate and invertebrate immune systems and argue that once bacteria can overcome the invertebrate immune system, they can more readily overcome the vertebrate immune system. We will also examine specific cases (*Tersinia, Bacillus cereus* and *Photorhabdus*) where putative arthropod vectors have facilitated the leap from invertebrates to vertebrates. We predict that as we learn more about the natural history of pathogens outside of man, that the origin of pathogenicity within invertebrates and its vectoring to man will be common.

Diseases of marine invertebrates

Symposium. Monday, 2:00.

*Hematodinium* sp.: Emergent pathogens for several commercial species of marine crustaceans

Theodore R. Meyers

Alaska Department of Fish and Game, Commercial Fisheries Division, P.O. Box 25526, Juneau, AK 99802, USA

Although *Hematodinium* sp. was described as early as 1931 in the green shore crab (*Carcinus maenas*) and harbor *Porinus depurator*) crabs, more recently this parasite has emerged as a significant pathogen in several other commercially important crustacean hosts from various oceans of the world. Approximately 20 species of crustacean hosts and 13 species of benthic amphipods worldwide have been parasitized by *Hematodinium* sp. Recent epizootics of *Hematodinium* sp. have been reported from Tanner crabs (*Chionoecetes bairdi, C. opilio*) in Alaska, USA and Newfoundland, Canada; the Norway lobster (*Nephrops norvegicus*) in Scotland; the velvet swimming crab (*Necora puber*) in France; the edible crab
(Cancer pagurus) in the UK and the blue crab (Callinectes sapidus) off the eastern coast of the USA. With the advent of molecular technology at least three species of Hematodinium have been proposed with a possible fourth based on morphological observations. The dinoflagellate causing Bitter Crab Syndrome (BCS) in Alaskan Tanner crabs is used to illustrate the features typical of Hematodinium sp. regarding host pathology, parasite morphology and potential economic losses caused by the disease. Also, there are likely management strategies for BCS that may be applicable to controlling Hematodinium epizootics in other populations of commercially important crab species.

Symposium. Monday, 2:30. 6
Herpesviruses infecting bivalves
Tristan Renault
IFREMER - Laboratoire de Génétique et Pathologie - 17390 La Tremblade, France

Particles morphologically similar to herpesviruses were first detected in an invertebrate (the Eastern oyster, Crassostrea virginica) by Farley et al. (1972). Herpes-like viruses have since been identified in various marine bivalve species throughout the world, including the Pacific oyster Crassostrea gigas, European flat oyster Ostrea edulis, Antipodean flat oyster Ostrea angasi, chilean oyster Tostrea chilenis, carpet shell clam Ruditapes decussatus, Manila clam Ruditapes philippinarum and great scallop Pecten maximus. Infections are often associated with sporadic episodes of high mortality among larvae and juveniles. PCR-based diagnostic methods have facilitated epidemiological investigations, for example showing that healthy adult animals can harbour the viral genome. Transmission experiments have demonstrated the pathogenicity of the virus, and indicated that a single species is probably responsible for all the infections observed. The virus isolated from injected C. gigas larvae has been formally classified as a member of the Herpesviridae under the name ostreid herpesvirus 1 (OsHV-1). The capsid morphology and genome sequence of OsHV-1 have been studied in order to assess its phylogenetic status in relation to vertebrate herpesviruses. The conserved gene that comes closest to being herpesvirus-specific encodes the putative ATPase subunit of the terminase. However, the presence of a distantly related gene in bacteriophage T4 leaves open the possibility of convergent evolution. Nonetheless, similarities between in capsid structure and mechanisms of capsid maturation tip the balance of evidence in favour of a common origin.

Symposium. Monday, 3:00. 7
Characterization of Perkinsus spp. and oyster herpes-like virus found in oysters collected in China, Japan and Korea
Kimberly S. Reece1, Jessica A. Moss1, Nancy A. Stokes1, Ryan B. Carnegie1, Christopher Dungan2 and Eugene M. Burreson1
1Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062, and 2Maryland Department of Natural Resources, Cooperative Oxford Laboratory, Oxford, MD 21654, USA

Decline of native Crassostrea virginica populations in the US mid-Atlantic states due to overfishing, disease, and habitat destruction, along with continued heavy disease pressure has led to interest in using the Asian oyster, Crassostrea ariakensis, for aquaculture development, fishery resource enhancement and habitat restoration in the Chesapeake Bay region. We conducted a disease survey of C. ariakensis in China, Japan and Korea. Molecular diagnostic screening using PCR based assays has revealed the presence of DNA from two Perkinsus species not currently found in U.S. waters; Perkinsus olseni and an undescribed Perkinsus species. Oyster herpes-like virus (OsHV) DNA also has been detected in oyster samples collected from potential broodstock sites in Asia. We are conducting studies to determine the potential impact these non-native pathogens could have on local bivalve species in case of accidental introduction with the host, or through another source such as ballast water, in which case C. ariakensis might act as a reservoir. Live oyster samples from southern China were recently brought into quarantine at our laboratory in order to further characterize the pathogens and to develop in vitro cultures of Perkinsus species. Challenge and transmission studies with the Asian Perkinsus spp. and OsHV are currently underway.

Symposium. Monday, 3:30. 8
Withering Syndrome, a rickettsial disease of abalone, Haliotis spp.
Carolyn S. Friedman
School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, WA 98195, USA

Catastrophic declines in many abalone species in California, both wild and cultured, have been attributed to the bacterial disease, Withering Syndrome (WS). The etiological agent was recently described as a novel rickettsia, “Candidatus Xenohaliotis californiensis” which infects gastrointestinal cells of abalone and results in morphologic changes in the digestive gland (degeneration and metaplasia/duct proliferation) and foot muscle (atrophy). The proliferative changes appear pathognomonic for WS. Differences in susceptibility of multiple species (Ostrea angasi, Haliotis cracherodii, and white, H. sorenseni, abalones are more susceptible to WS than are red, H. rufescens, and green, H. fulgens, abalones. Climatic variation associated with ENSO events has been demonstrated to result in development of WS in black, red, white and green abalones. Molecular tools (in situ hybridization, PCR and Q-PCR tests) and treatments (oxytetracycline, OTC) have been developed. The digestive gland of OTC-mediated abalone concentrates the drug resulting in slow depletion (> 6 mo.) thus providing long term protection from re-challenge (~2.5-5.5 mo). These tools will play a key role in the abalone culture industry and captive broodstock programs, particularly for the endangered white abalone, H. sorenseni, which is being cultured in a WS endemic region.

Fungi

CONTRIBUTED PAPERS. Monday, 2:00-4:00

Susceptibility of four native lady beetle species to Beauveria bassiana
Ted E. Cottrell and David I. Shapiro-Ilan
USDA, Agricultural Research Service, SE Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008, USA

Little is known about differential susceptibility of lady beetle species to entomopathogens and how these entomopathogens might affect lady beetle populations in the field. Previous research showed that a lady beetle species native to North America, Olla v-nigrum, was commonly found infected by Beauveria bassiana in the field. Laboratory assays showed that this native lady beetle was susceptible to the endemic strain of B. bassiana whereas, an exotic lady beetle, Harmonia axyridis was not. We hypothesize that differential susceptibility of native and exotic lady beetles to entomopathogens has facilitated the establishment of the exotic H. axyridis across North America. Here we have used laboratory assays to test the susceptibility of three other native North American lady beetle species (Coleomegilla maculata, Hippodamia convergens and Cycloneda munda), along with O. v-nigrum, to two strains of B. bassiana. Mortality of H. convergens, C. munda and O. v-nigrum was always highest when tested against B. bassiana isolated from O. v-nigrum whereas, C. maculata was not. Beetles assayed with the commercially-available GHA strain, and control beetles, had similar lower mortality.
The British winter represents adverse conditions for ladybirds: aphids are in scarce supply and climatic conditions are unfavourable. Therefore, ladybirds spend the winter months in a dormant state. Cocinella septempunctata (seven-spot ladybird) and Adalia bipunctata (two-spot ladybird) are the two most common species of ladybird in Britain. The fungal pathogen Beauveria bassiana is often reported as being a major mortality agent of overwintering seven-spot ladybirds but not of two-spot ladybirds. It is assumed that this is related to the different overwintering strategies employed by these two ladybird species. Seven-spot ladybirds commonly spend the winter in leaf litter, where the prevalence of B. bassiana is high. In contrast, two spot ladybirds overwinter in above ground positions, such as trees or buildings, and so have limited contact with the fungus. However, it could also be that two-spot ladybirds are not susceptible to B. bassiana. In this paper we present results of laboratory experiments which confirm that both seven-spot and two-spot ladybirds are susceptible to B. bassiana but that susceptibility is reduced significantly after the ladybirds have been exposed to cold temperatures for one month. We discuss the ecological and evolutionary significance of these results and suggest physiological mechanisms that could be involved.

**Effect of in vivo passage of Beauveria bassiana through aphid versus non-aphid hosts on the relative virulence towards two cereal aphid species (Homoptera: Aphididae)**

Justin L. Hatting

ARC-Small Grain Institute, P/Bag X29, Bethlehem, 9700, South Africa

The two principal aphid pests of dryland-produced wheat in the summer and winter rainfall regions of South Africa are Russian wheat aphid, Diuraphis noxia, and oat aphid, Rhopalosiphum padi, respectively. South African surveys have revealed several entomophthoralean fungi infecting these species although the level of mycosis never exceeded 5 percent in R. padi compared with up to 50% in D. noxia. Moreover, infection by the hymenomycetous fungi, Beauveria bassiana and Leccanilium lecanii, was observed only in D. noxia collected from the winter rainfall region. These observations suggest some level of low susceptibility to fungal infection in R. padi. Previous assays with four strains of B. bassiana against D. noxia have shown high susceptibility with mortalities ranging from 71.7±27.1 to 97.8±3.4% at an average application rate of 2030±47.78 conidia per mm². The purpose of this study was to verify a lower level of susceptibility to fungal infection in R. padi and to investigate the effect of preconditioning of B. bassiana on the virulence towards a susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected B. bassiana strain (PPRI 7313) preconditioned on either with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected susceptible versus less-susceptible host. Both aphids were inoculated with a single maximum-challenge conidial-dose from a soil-collected.
Colorado potato beetle, Leptinotarsa decemlineata. Scanning electron microscopy revealed different in vivo behavior for unipolar- and bipolar-germinated conidia. Unipolar-germinated conidia created a strong germ tube with mostly appressorium-like structures while bipolar-germinated conidia continued to invasive hyphal growth without any penetration, indicating that germination polarity in one way or another may be an indicator of pathogenic ability.

Contributed paper. Monday, 3:30. 15

Virulence and fitness of the fungal pathogen Entomophaga maimaiga in its host Lymantria dispar, for pathogen and host strains originating from Asia, Europe and North America

Charlotte Nielsen¹, Melody Keena², and Ann E. Hajeck¹

¹Department of Entomology, Cornell University, Ithaca, NY 14853, USA, ²USDA Forest Service, Northeastern Research Station Hamden, CT 06514, USA

The European biotype of gypsy moth, Lymantria dispar, was introduced to the US from France in 1868 and has since become a major pest. In addition, the Asian biotype of gypsy moth has been accidentally introduced in the US, but so far eradication programs have been successful in preventing establishment of this biotype. We tested whether non-North American gypsy moth are susceptible to North American E. maimaiga isolates and the potential for erosion in the efficacy of E. maimaiga. We used bioassays to assess virulence and fitness of the pathogen in four gypsy strains challenged with six E. maimaiga isolates, using host and pathogen strains from Asia, Europe and the US. All E. maimaiga isolates tested were pathogenic to all strains of L. dispar. Fungal isolates differed significantly with regard to both virulence and fitness, whereas gypsy moth strain seemed to have little effect on fungal virulence and fitness. The similar patterns of virulence and fitness observed in the US and Asian gypsy moth populations challenged with E. maimaiga indicate that erosion of successful control of gypsy moth by E. maimaiga is unlikely based on our studies with laboratory-reared gypsy moth strains.

Contributed paper. Monday, 3:45. 16

Growth characteristics and virulence of insect pathogenic fungi at low temperatures

Linda Hjeljord¹ and Ingeborg Klingen²

¹Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Aas, Norway, ²The Norwegian Crop Research Institute, Hogskolevn. 7, N-1432 Aas, Norway

In an effort to identify isolates of insect pathogenic fungi showing high virulence in a cool environment, studies were conducted to investigate the effect of temperature on germination speed, growth rate, competitive ability and virulence of strains isolated from different latitudes in Norway. A total of 13 strains of, including Beauveria bassiana (Bb), Metarhizium anisopliae (Maj), Paecilomyces farinosus, and Lecanicillium lecanii, were tested at temperatures from 6-37°C. Virulence of selected isolates was tested in bioassays at three relevant temperatures (6, 12, and 18 oC) against Colorado potato beetle, Otiorhynchus sulcatus (Coleoptera), Operophthera brumata (Lepidoptera), and Mamestra brassicae (Lepidoptera). Results showed that the insect pathogens had strain-specific germination rates. The fastest of the Bb strains germinated significantly (p<0.001) faster than the slowest at temperatures from 12°C - 30°C. The difference in germination rates between the fastest and the slowest Ma strains was consistent, but less statistically significant (p=0.055). Ma strains germinated more slowly than Bb strains, especially at low temperatures (e.g. 50% germination time at 12°C was 35.2 ±3.5 h for Bb strains, and 69.9 ±22.2 h for Ma strains). Bb strains were more competitive than Ma strains at temperatures >21°C. There was no clear correlation between germination rate and virulence in the isolates selected for the bioassays, perhaps due to the relatively few isolates tested.
anisotropy and quantum yield, but an unchanged lifetime, which is consistent with the presence of toxin aggregates in the membrane. Electrostatic interactions between the charged amino acid residues and the lipid head groups are responsible for bringing the protein to the membrane surface, while hydrophobic and/or van der Waals interactions make the membrane binding irreversible. Fluorescence photobleaching recovery, a technique that measures diffusion coefficient of fluorescently labeled particles, and epifluorescence microscopy revealed that upon addition of Cry1A lipid vesicles were broken into smaller, faster diffusing objects. Since no change in size or morphology of the vesicles is expected when pores are formed in the osmotically equilibrated membranes, our results support the detergent-like mode of action of Cry1A.

**STU** Contributed paper. Monday, 2:45  
**Protease inhibitors fail to prevent pore formation by the activated Bacillus thuringiensis toxin Cry1Aa in insect brush border membrane vesicles**  
Martin Kirouac, Vincent Vachon, Delphine Quevry, Jean-Louis Schwartz and Raynald Laprade

Groupe d’étude des protéines membranaires, Université de Montréal, P.O. Box 6128, Centre Ville Station, Montreal, Quebec, H3C 3J7, and Biocontrol Network, Canada

To investigate the possible involvement of membrane proteases in the activity of *Bacillus thuringiensis* insecticidal toxins, the rate of pore formation by trypsin-activated Cry1Aa was monitored, in the presence of a variety of protease inhibitors, with *Manduca sexta* midgut brush border membranes and a light-scattering assay. Most of the inhibitors tested had no effect on the pore-forming ability of the toxin. However, phenylmethylsulfonyl fluoride, a serine protease inhibitor, promoted pore formation. Although this result is consistent with the presence of a membrane protease that could interact with the toxin and reduce its activity, several other serine protease inhibitors were ineffective. Among the metalloprotease inhibitors, o-phenanthrolone had no significant effect while EDTA and EGTA reduced the rate of pore formation at pH 10.5, but only EDTA was inhibitory at pH 7.5. Neither chelator affected the properties of the pores already formed after incubation of the vesicles with the toxin. Taken together, these results indicate that once activated Cry1Aa is completely functional. The effect of EDTA and EGTA is probably better explained by their ability to chelate divalent cations that could be necessary for the stability of the toxin’s receptors or involved elsewhere in the mechanism of pore formation.

**STU** Contributed paper. Monday, 3:00  
**Differential effects of ionic strength and pH on the pore-forming activity of Bacillus thuringiensis insecticidal toxins**  
Mélanie Fortier, Martin Kirouac, Vincent Vachon, Olivier Peyronnet, Jean-Louis Schwartz and Raynald Laprade

Groupe d’étude des protéines membranaires, Université de Montréal, P.O. Box 6128, Centre Ville Station, Montreal, Quebec, H3C 3J7, and Biocontrol Network, Canada

The lepidopteran midgut lumen is characterized by a highly alkaline pH and a high ionic strength, two factors which are expected to modulate electrical charges at the cell surface and therefore influence the interaction of *Bacillus thuringiensis* toxins with the insect epithelial cell membrane. The combined effects of ionic strength, pH, divalent cations and ionic strength on the pore-forming activity of Cry1Ac and Cry1C were therefore studied using membrane potential measurements in isolated midguts of *Manduca sexta* and a brush border membrane vesicle osmotic swelling assay. The effects of ionic strength and divalent cations were most pronounced at pH 10.5. In isolated midguts, lowering ionic strength enhanced Cry1Ac activity but decreased considerably that of Cry1C. In vesicles, the activity of Cry1C, which was small at low ionic strength, was greatly increased by adding calcium or by increasing ionic strength. EDTA inhibited Cry1Ac activity, indicating that divalent cations are necessary for Cry1Ac activity. These results, which clearly demonstrate a strong effect of pH, ionic strength and divalent cations on the pore-forming activity of Cry1Ac and Cry1C, stress the importance of electrostatic interactions in the mechanism of pore formation by *B. thuringiensis* toxins.

**STU** Contributed paper. Monday, 3:15  
**Mode of action of Bacillus thuringiensis insecticidal toxin Cry9Ca: Effect of the physico-chemical microenvironment on pore formation in Manduca sexta intestinal membranes**  
Jean-Frédéric Brunet1, Vincent Vachon1, Mireille Mansolias1, Jeroen van Rei2, Jean-Louis Schwartz2 and Raynald Laprade1

1Groupe d’étude des protéines membranaires, Université de Montréal, Montréal, Québec and Biocontrol Network, Canada, 2Bayer BioScience NV, Ghent, Belgium

Once ingested by susceptible insects, *Bacillus thuringiensis* insecticidal toxins must be transformed into an active form by the insect’s intestinal proteases. Their first domain, a bundle of amphipathic α-helices, is responsible for their insertion into the luminal membrane of midgut cells, thereby creating poorly selective pores. In the case of Cry9Ca however further hydrolysis forms a 55-kDa fragment previously reported as being non toxic. The same study had shown that mutant R164A was not subject to this degradation event. Wild-type Cry9Ca, mutants R164A and R164K and the 55-kDa fragment successfully depolarize membranes of freshly isolated *Manduca sexta* midguts bathing in a pH 10.5 and high ionic strength solution. This depolarization is especially rapid with the 55-kDa fragment. For Cry9Ca and both mutants, the depolarization is strongly enhanced by protease inhibitors or intestinal juice. Neither denaturing its proteins at 95°C nor reducing its ionic strength through dialysis abolished the stimulatory effect of intestinal juice. Toxin activity was also enhanced when intestinal juice was replaced by its lipids. These results indicate that pore formation by Cry9Ca is strongly dependent on the physico-chemical conditions under which it occurs and stress the importance of additional proteolysis sites on the toxin molecule.

**STU** Contributed paper. Monday, 3:30  
**Directed mutagenesis of conserved aromatic residues in helix 7 critical for larvicidal activity of the Bacillus thuringiensis Cry4Ba toxin**  
Kasorn Tiewsiri and Chanan Angsuthanasombat

Laboratory of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand

The detailed information of the highly conserved helix 7 in the pore-forming domain of the *Bacillus thuringiensis* Cry δ-endotoxins remains to be investigated. In the present study, alanine substitutions of three conserved aromatic residues in α7 (Phe-246, Tyr-249 and Phe-264) of the Cry4Ba mosquito-larvicidal protein were performed via PCR-based mutagenesis. All the mutant toxins were highly expressed in *Escherichia coli* as 130-kDa protoxins at levels comparable to the wild-type toxin. Bioassays against *Aedes aegypti* mosquito larvae revealed that *E. coli* cells expressing Y249A or F264A mutant toxins displayed a dramatic decrease in toxicity, but not for the F246A mutant. Further mutagenesis analysis showed that only replacements with an aromatic residue, Y249F or Y249W and F264Y or F264W, still retained the high level of toxicity, while substitutions with Glu, Arg or His almost completely abolished larvicidal activity. These results suggested that aromatic side-chains of these two critical residues, Tyr-249 and Phe-264, within helix 7 of the Cry4Ba toxin play an important role in larvicidal activity.

**STU** Contributed paper. Monday, 3:45  
**Mutagenic analysis of the transmembrane helix 5 of the Bacillus thuringiensis Cry4Ba toxin reveals a crucial role in larvicidal activity for Asn-183**  
Supaporn Likitvivatanavong and Chanan Angsuthanasombat

Laboratory of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand
The proposed toxicity mechanism of the Bacillus thuringiensis Cry δ-endotoxins involves the penetration of α-helices 4 and 5 to form lytic pores in the target cell membrane. In this study, alanine substitutions of selected polar residues (Tyr-178, Gln-180, Asn-183, Asn-185 and Asn-195) in the relatively hydrophilic transmembrane α5 of the Cry4Ba mosquito-larvicidal protein were initially conducted via PCR-based directed mutagenesis. Upon IPTG induction, all mutant proteins were highly expressed in Escherichia coli as cytoplasmic inclusions, with yields similar to the wild-type protoxin. When E. coli cells expressing each mutant toxin were tested against Aedes aegypti mosquito larvae, the larvicidal activity was almost completely abolished for the N183A mutation, whereas the other four mutant toxins showed only a small reduction in toxicity. Further replacements of this critical residue with various amino acids revealed that the polarity at position 183 of the transmembrane α5 play a crucial role in larvicidal activity of the Cry4Ba toxin.

Symposium, Monday, 5:08.  27
Use of pathogens against incursion pests in New Zealand
Travis R. Glare1 and Ian R. Gear2

New Zealand is an island nation with unique indigenous flora and fauna. Recent history has demonstrated that the exclusion of exotic insect species is important to maintain the health of natural ecosystems and economic production in this agricultural-based country. In the last decade incursions of four lepidopteran species, the white spotted tussock moth, Orgyia thyellina, the painted apple moth, Teia arantoides, gypsy moth, Lymantria dispar, and, most recently, fall webworm, Hyphantria cunea, have been discovered. Eradication programmes have been undertaken using a range of tools including delimiting surveys, trapping, ground searches and aerial operations utilising Bacillus thuringiensis kurstaki. Eradication has been declared for white spotted tussock moth and Asian gypsy moth. Painted apple moth is timetabled to be declared eradicated in January 2006. These programmes, led by the Ministry of Agriculture and Forestry (MAF), involved many areas of operation in addition to the pest control aspects, such as economic and environmental impact assessments, human health risk assessments, health surveillance and monitoring studies, and stakeholder communications. In addition, eradication projects (using S-methoprene and B. thuringiensis israelensis) are being conducted by the Ministry of Health for the exotic salt marsh mosquito (Ochlerotatus camptorhynchus), a potential vector for Ross River virus disease.

Symposium, Monday, 5:32.  28
Development of fungal bands to assist in eradication of Asian longhorned beetle, Anoplophora glabripennis, in the U.S.
Ann E. Hajek1, James R. Reilly1, Thomas Dubois3, Michael Smith3, Leah Bauer1 and Zengzhi Li1

1Department of Entomology, Cornell University, Ithaca, NY 14853-0901, USA, 2USDA, ARS, BHRL, Newark, DE 19713-3814, USA, 3USDA, Forest Service, East Lansing, MI 48823-5286, USA, and 4Department of Forestry, Anhui Agricultural University, Hefei, Anhui 230036, China

Non-woven fiber bands impregnated with cultures of entomopathogenic fungi have been investigated for control of the cerambycid, Anoplophora glabripennis (Asian longhorned beetle), which was introduced from China to several locations in the U.S. This beetle was first found in the New York City area in 1996 and is presently the focus of an eradication campaign based on intensive efforts to detect beetles and application of imidacloprid to soil or tree trunks, although neither of these methods are one hundred percent effective. The additional control method we are developing, application of fungal bands to trees, relies on self-inoculation by adult beetles, especially during the pre-maturational wandering period which lasts 1-2 weeks after eclosion. In addition, inoculated beetles can transfer conidia to the opposite sex during mating. Field trials conducted at sites in China where fungal bands were attached to all trees in treatment plots demonstrated both decreased adult longevity and decreased oviposition in fungal treatment plots. Bands placed on trees in Queens, New York retained high conidial densities for over 3 months. Using data we have developed on the effects of fungal bands on beetle populations, we created simple models to investigate the impact of fungal bands toward eradication of A. glabripennis under different estimates of A. glabripennis density and different threshold densities of beetles required for an Allee effect.

Symposium, Monday, 4:44.  26
Assessing short-term human health effects of Bacillus thuringiensis applied during insect control programs
David B. Levin
Department of Biology, University of Victoria, British Columbia, V8W 3N5, Canada

There is considerable evidence that Bacillus thuringiensis (Bt) is neither toxic, nor pathogenic to mammals. None-the-less, applications of Bt sprays in populated urban centers generates considerable public concern about the impact of exposures to Bt on human health. Aerial applications of Foray 48B, which contains Bt subspecies kurstaki, strain HD1 (Btk HD1), were applied to control the European gypsy moth (Lymantria dispar) in Victoria, British Columbia, Canada, in 1999. An assessment of the health impact of Foray 48B was also conducted during this period. Environmental and human samples, collected before and after aerial applications of Foray 48B, were analyzed for the presence of Bt HD1-like bacteria. Molecular methods were used to determine the identity of over 11,000 isolates from environmental and human samples. Several health indicators, including an assessment of the impact of the spray on asthmatic children, we measured. Results of the studies suggest that Bt HD1-like bacteria were present both in the environment and in the human population of Victoria prior to aerial applications of Foray 48B, and that the spray had no detectable adverse effect on the human population.
**Varroa mite control with fungal pathogens: Will this little piggy get to market?**

Rosalind R. James

USDA-ARS Pollinating-Insect Research Unit, Logan, UT, USA

*Varroa destructor* is a mite parasitic to honey bees, causing severe economic damage due to its impact on both honey production and pollination services. Varroa has developed wide-spread resistance to commonly used miticides, and these chemicals have recently been discovered to negatively affect the bees. If a biological control strategy were readily available, a high percentage of the beekeepers would probably adopt it. We screened known mite pathogens, rather than searching for new pathogens specific to varroa, under the hypothesis that a pathogen from another host might yield the highest virulence. In addition, this strategy allowed us to focus on indigenous fungal strains. Some strains of *Hirsutella thompsonii* and *Metarhizium anisopliae* were found to have high pathogenicity. *H. thompsonii* was a poor candidate in the field, however, possibly due to qualities associated with the spore. *M. anisopliae* is easy to mass produce and yielded good results in the field. The greatest hurdle to this research has been to find a commercial enterprise adequately equipped to bring this product to the market. The beekeeping market is small, and some of the infrastructure necessary to produce a product is currently lacking, so investment opportunities are limited.

**Symposium. Monday, 5:56. 29**

**Varroa mite control with fungal pathogens: Will this little piggy get to market?**

**Pathogen diversity and the efficacy of virus insecticides**

Jenny S. Cory1,2, David J. Hodgson1 and Elizabeth M Redman1

1Ecology and Biocontrol Group, NERC Centre for Ecology and Hydrology, Mansfield Rd, Oxford OX1 3SR, UK, 2Alice University College, University of Oxford, 1520 Queen Street East, St. Albans Sainte Marie, Ontario, P6A 2G4, Canada and 3School of Biological and Chemical Sciences, University of Exeter, Hatherley Laboratories, Prince of Wales Rd, Exeter EX4 4PS, Devon, UK

It is becoming increasingly apparent that baculovirus populations are genetically diverse and that insect hosts are commonly infected by multiple genotypes. The nature and outcome of interactions between parasite genotypes within hosts will determine the severity and spread of disease, with implications for the use of pathogens in pest management campaigns. Using several lepidopteran-NPV systems, we have shown that infections comprising more than one genotype are more pathogenic, on average, than single variant infections. We have also explored the impact that mixed infections have on the trade-off between virus productivity and speed of kill and that, under certain circumstances, high yield can be maintained. However, virus dose can also have a significant impact on the outcome of these mixed infections. These results demonstrate that retention of genotypic diversity in bioinsecticide applications can benefit pest management campaigns.

**STU Contributed paper. Monday, 4:35. 31**

**The role of viral pathogens in the regulation of lepidopteran host populations: The winter moth and its natural enemies**

Robert L. Graham1, Shujing Rao2, Steven M. Sait2, Robert D. Possee1, Peter P. C. Mertens2 and Rosemary S. Hails1

1NERC Centre for Ecology and Hydrology, Mansfield Road, Oxford, OX1 3SR, 2Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, 3Ecology and Evolution Research Group, School of Biology, University of Leeds, Leeds, LS2 9JT, UK

A lepidopteran system is reported, in which viral pathogens are both abundant and genotypically variable. Geographically separate populations of Winter moth (*Operophtera brumata*) L were sampled in heather habitats over three years to investigate the prevalence of four viral pathogens, and to evaluate their role in regulating the host lepidopteran populations. A Nucleopolyhedrovirus (OpbuNPV) was recorded in eleven of the thirteen winter moth populations sampled, with two populations suffering viral mortality at rates of over 50%. Restriction endonuclease and sequence analysis has allowed the discovery of 41 genotypically different strains of OpbuNPV. Three species of reovirus have been detected, all of which are novel. Electron microscopy and sequence data place two of the viruses within the *Cypoviridae* genus (CPV). The third reovirus was isolated from both the Winter moth and a hymenopteran parasitoid wasp (*Phobocampse tempestiva*). Sequence data classifies this virus as an unspecified reovirus. The relationship between the NPV and the CPV is investigated, as well as the impact of viral pathogens on the host lepidopteran populations. The transmission strategies of these viruses are presented, discussing the role of vertical transmission and parasitoid vectoring. This research has provided a significant insight into the interactions of pathogens and parasitoids in the regulation of lepidopteran populations.

**STU Contributed paper. Monday, 4:50. 32**

**Investigating the genetic parameters that affect virus transmission**

Fiona L. King1, Rosemary S. Hails2, Robert D. Possee1, Linda A. King1

1School of Molecular and Biological Sciences, 2Department of Biological Sciences, Oxford Brookes University, Oxford, OX3 0BP, 3Centre for Environment and Environmental Microbiology (CEH), Oxford, OX1 3SR, UK

Virus transmission is fundamentally important for virus survival. Heat, light and desiccation can affect the sustainability of virus infectivity, especially when the virus is outside its normal host. The *Baculoviridae* family are a group of insect-specific viruses separated into two genera: nucleopolyhedrovirus (NPVs) and granuloviruses (GVs). Virus infection has been described in detail for NPVs, where the final stages are characterised by host liquefaction. Liquefaction ensures efficient virus transmission between hosts. The ability for NPVs to induce liquefaction has been linked to two virally encoded enzymes, chitinase and protease. Insects contain chitin-rich areas for strength and support. During the course of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) infection, these enzymes are hypothesised to work in synergy to degrade chitin, inducing host liquefaction. Published work has shown that removal of either chitinase and/or cathepsin can disrupt host liquefaction. The focus of this research is to determine how the removal of either chitinase and/or cathepsin affects virus transmission. Results to date demonstrate removal of either gene significantly affects virus lethality and speed of kill. Host liquefaction guarantees a rapid transmission rate. Meanwhile lack of host liquefaction occurs at a reduced rate, but with no detriment to transmission. Ultimately, if a model can be produced to demonstrate the parameters required for efficient transmission of these insect-specific viruses, the knowledge gained may aid development of baculoviruses as a biopesticide.

**Contributed paper. Monday, 5:05. 33**

**Biological and molecular characterization of iranian-caucasian isolates of *Cydia pomonella* granulovirus (CpGV)**

S. Sayed1,2, M. Rezapahan1, S. Shoja-I-Estrabragh1 and L. A. Jehle1

1Laboratory of Biotechnological Crop Protection, Agricultural Service Center Palatinate, Neustadt/Wstr., Germany, 2Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Egypt, 3Biocontrol Research Dept., Plant Pests and Diseases Research Institute, Tehran, Iran, 4National Research Center of Genetic Engineering & Biotechnology, Tehran, Iran

The codling moth, *Cydia pomonella* is the most serious insect pest of pomaceous fruits, especially of apples and pears. *C. pomonella* granulovirus (CpGV) is a viral pathogen of the codling moth larvae, which is registered and widely used in Europe for controlling this pest in apples. Six CpGV isolates from different geographical regions in Iran and two others obtained from Georgia were studied in order to analyze the biological and molecular differences between these isolates. The isolates were propagated in 5th instar of codling moth. Viral DNA was isolated and characterized by endonuclease restriction
analysis using BamHI, EcoRI, PstI and XhoI. Differences between the isolates were identified and mapped in correspondence to the Mexican isolate of CpGV. The obtained results indicated that some of these isolates were related to CpGV-M (Mexican isolate), CpGV-E (English isolate) or CpGV-R (Russian isolate). However, these isolates showed a considerable molecular diversity. Bioassays (median lethal concentration LC50 and median survival time ST50) were determined for newly hatched C. pomonella larvae with all isolates along with CpGV-M as a control. The molecular and biological characteristics of these isolates will be presented.

**STU** Contributed paper. Monday, 5:20. 34

Enhancement in activity of Turkish SpliNPV-B to Spodoptera littoralis Boisid. (Lepidoptera:Noctuidae) by an optical brightener

Umut Topraķ and Oktay Gürkan

University of Ankara, Faculty of Agriculture, Department of Plant Protection, 06110 Dışkapı/Ankara/Turkey

Previous studies have shown that certain optical brighteners are effective UV protectants and enhance viral activity in some baculovirus-lepidopteran host systems. In our study, enhancement in activity of SpliNPV-B to 3rd instar Spodoptera littoralis Boisid. (Lepidoptera: Noctuidae) larvae by an optical brightener was evaluated by a lettuce leaf disk assay. In the absence of 1 % Tinopal UNPA-GX, LD50 of SpliNPV-B was found 72.95 polyhedra. In the case of addition of 1 % Tinopal UNPA-GX to the virus suspension, LD50 decreased to 5.65 polyhedra, indicating that Tinopal UNPA-GX increased larval susceptibility to NPV infection by 12.9 times. On the other hand, the mean time to death was not affected by the addition of 1 % Tinopal UNPA-GX for third instars at a dose of 3000 polyhedra.

Contributed paper. Monday, 5:35. 35

Nutritional self-medication by insects in response to protein costs of virus resistance

Kwang Pum Lee1,2, Jenny S. Cory1, Kenneth Wilson2, David Raubenheimer1 and Stephen J. Simpson1

1Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK, 2Department of Biological Sciences, Institute of Environmental and Natural Sciences, University of Lancaster, Lancaster LAI 4YQ, UK and 3Molecular Ecology and Biocontrol Group, NERC Centre for Ecology and Hydrology , Mansfield Road, Oxford OX1 3SR, UK

Mounting effective resistance against pathogens is costly in terms of energy and nutrients. However, it is unclear whether hosts can offset these costs by adjusting their dietary intake so as to recoup the specific resources involved. We test this possibility by experimentally challenging a generalist-feeding caterpillar (Spodoptera littoralis), with dietary regimes varying in the content of protein and digestible carbohydrate. We find that dietary protein influences both virus resistance and constitutive immune function of a caterpillar to a greater extent than dietary carbohydrate. This indicates that mounting resistance costs more in protein than energy. Moreover, when the larvae were allowed to select their nutrient intake, insects surviving viral challenge increased their relative intake of protein compared with untreated controls and those dying of infection, thus demonstrating compensation for protein costs associated with resistance. Our results suggest that the change in the host’s nutritional demands in order to fight infection induces a compensatory shift in feeding behaviour, and provide first experimental evidence for “nutrient self-medication” in animals.

Contributed paper. Monday, 5:50. 36

Disruption of climbing behavior prior to death of gypsy moth (Lymantria dispar) larvae infected with egt-deletion constructs of LdNPV

Mike Grove1, Brianna Reed1, A. Daniel Jones1, Nancy Hayes-Plazollo3, James Slavicek4 and Kelli Hoover4

1The Pennsylvania State University Department of Entomology 501 A.S.I. Building University Park PA 16802 and 2 USDA Forest Service, 359 Main Road Delaware, Ohio 43015, USA

Baculovirus-infected gypsy moth larvae typically climb upwards immediately before dying, assuming elevated positions similar to those taken by larvae preparing to molt to the next instar. We hypothesized that fluctuating edysone titers might control both behaviors. Inactivation of edysone by the egt gene product, an enzyme normally produced by baculoviruses, might induce abnormal climbing behavior in infected gypsy moth larvae. To test this hypothesis, we inoculated larval cohorts with an assortment of wild-type; β-galactosidase-expressing; egt-deleted; and β-galactosidase-expressing, egt-deleted baculoviruses. Deletion of the egt gene eliminated climbing behavior in both egt (-) constructs tested, whether β-gal was also expressed or not. However, one of three non egt deleted viral constructs, designated 7H5, also failed to induce normal climbing behavior. We are currently investigating if egt function of the 7H5 virus was unintentionally disrupted during transformation, or whether a second mechanism might be involved in LdNPV-induced climbing behavior.

Contributed paper. Monday, 6:05. 37

Effects of a protease-expressing recombinant baculovirus insecticide on the parasitoid Cotesia margariventris (Cresson)

Tyasning Nusawardani1, John R. Ruberson2, John, J. Obreyki1, and Bryony C. Bonning3

1Department of Entomology, Iowa State University, Ames, IA 50011, USA, 2Department of Entomology, University of Georgia, Tifton, GA 31793, USA, 3Department of Entomology, University of Kentucky, Lexington, KY 40546, USA

The recombinant baculovirus AcMLF9.ScahL expresses a basement membrane-degrading protease (ScahL) and kills lepidopteran larvae significantly faster than the wild type baculovirus. Risk assessment studies were conducted to examine the potential impact of the virus on the parasitoid Cotesia margariventris (Cresson) that parasitizes infected larvae. Larvae of Heliocis virescens were exposed to the parasitoid and infected with >LC99 dose of the wild type or recombinant virus at 72, 96, or 120 hours after parasitism. At 72 hr post parasitism, the survival of parasitoids emerging from hosts infected with wild-type virus. There were no significant differences between AcMLF9.ScahL and wild type virus treatments for larval and adult parasitoid emergence times, size, sex ratio, or fecundity. Virus infection did not affect parasitoid host choice. Virus was detected by PCR in approximately 35% of parasitoids that emerged from virus infected larvae, and these parasitoids were able to transmit virus to other hosts. Parasitism blocked ScahL-induced melanization of host larvae. These results indicate that AcMLF9.ScahL poses a slightly greater risk to the parasitoid than the wild type virus if infection of the host larva occurs at a high dose, 72 hours after parasitism.

CONTRIBUTED PAPERS. Monday, 4:20-6:20

MICROSPORIDIA AND PROTOZOA

Contributed paper. Monday, 4:20. 38

Competition between the microsporidia Nosema lymnaeae and Vairimorpha sp. parasitizing Lymantria dispar larvae: The importance of timing for successful establishment and horizontal transmission of infection

Daniela Pilarska1, Leellen F. Solter2, Andreas Linde1, Manana Kereselidze3 and Gernot Hoch4

1Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 2Center for Economic Entomology, Illinois Natural History Survey, Champaign, IL, USA, 3Department of Forestry, University of Applied Sciences, Eberswalde, Germany, 4V. Gulisashvili Institute of Mountain Forestry, Academy of Sciences, Tbilisi, Republic of Georgia, 5Department of Forest and Soil Sciences, BOKU University of Natural Resources and Applied Life Sciences, Vienna, Austria

We studied competition between Nosema lymnaeae and Vairimorpha sp. in their natural host, the Lymantria dispar larva. Hosts were orally infected with the microsporidia, either simultaneously or with a time lag of 3 or 7 days between the
infections. Successful establishment in the larvae was evaluated by light microscopy and horizontal transmission of mixed pathogen infections to test larvae was quantified. Timing of the infections determined the outcome. At a 7–day interval between challenges, the microsporidian species inoculated first almost completely excluded the second. In cases of simultaneous infection, establishment of neither species was reduced significantly. These results were reflected in transmission; neither Nosema nor Vairimorpha prevalence was significantly lower in test larvae compared to single-pathogen infections. When the time interval between inoculations was 3 days, mixed infections occurred frequently, especially when Nosema was administered first. When Vairimorpha was administered before Nosema, Vairimorpha was clearly dominant and frequently excluded Nosema. This situation was also reflected in transmission of the two microsporidia. Establishment of Vairimorpha in test larvae did not differ significantly between single-pathogen infections and mixed infections in initially-infected larvae. Transmission of Nosema was negatively affected by competition with Vairimorpha.

Contributed paper. Monday, 4:35. 39

**Effects of Nosema fumiferanae (Microspora) on distribution and dispersal of overwintering spruce budworm larvae**

Kees van Frankenhuyzen and Carl Nystrom

Great Lakes Forestry Centre, Canadian Forest Service, 1219 Queen street East, Sault Ste. Marie, Ontario, P6A 2E5, Canada

*Nosema fumiferanae* is a widespread pathogen in epidemic populations of the spruce budworm. Chronic infection debilitates fitness by retarding larval development, and reducing adult size, longevity, and fecundity. Direct mortality occurs only at high infection intensities. We postulated that *Nosema* infection might affect distribution of overwintering larvae and subsequent dispersal in the spring. Field studies were conducted on mature white spruce in Prince Albert National Park, Saskatchewan. Infection by *Nosema* did not affect the distribution of overwintering larvae. There were only small differences in *Nosema* prevalence in larvae between upper-, middle- and lower-canopy branches, or between branch tips versus the rest of the branch. In contrast, infection had a pronounced effect on emergence of larvae from their hibernaculae and subsequent dispersal in the spring. When branches were forced inside, diseased larvae emerged later than healthy larvae. In the field, diseased larvae tended to disperse later than healthy ones, as was demonstrated by trapping dispersing larvae on sticky traps suspended between tree crowns. Later dispersal resulted in loss of infected larvae from the population. Our results suggest that indirect mortality caused by *Nosema* needs to be considered when evaluating its role in spruce budworm population dynamics.

Contributed paper. Monday, 4:50. 40

**Effect of microsporidia on the life history of the convergent lady beetle, Hippodamia convergens (Guérin-Méneville)**

Philip Joudrey

Department of Biology, Saint Mary’s University, Halifax, Nova Scotia, B3H 3C3, Canada

Although microsporidia were first discovered to infest the lady beetle *Hippodamia convergens* (Guérin-Méneville) in 1959, the effects of microsporidia on this coccinellid have not been studied. In North America, *Hippodamia convergens* is the primary lady beetle used in biological control. Billions of these coccinellids are collected from overwintering sites in California and sold by commercial insectaries throughout the United States and Canada. The focus of this project was to investigate the effects of microsporidia on fecundity, egg viability, longevity, larval development and sex ratio of commercially available *H. convergens*. Microsporidia infected and uninfected beetles were reared individually from egg to adult and through to death on an *ad libitum* diet of green peach aphids (*Myzus persicae* Sulzer). Larvae were checked daily for signs of moulting and adults were checked daily for egg production. Eggs were kept for one week to observe egg hatch. Upon death, beetles were smeared, stained and examined for the presence of microsporidian spores.

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**Microsporidia in Hippodamia convergens (Guérin-Méneville) used for biological control in agroecosystems**

Susan Bjornson

Department of Biology, Saint Mary’s University, Halifax, Nova Scotia, B3H 3C3, Canada

Convergent lady beetles (*Hippodamia convergens* Guérin-Méneville) are often collected from hibernation sites in California and shipped to commercial insectaries, then to commercial growers and home gardeners who release them for localized aphid control. Unfortunately, beetles collected from hibernation sites have a tendency to disperse upon release and there is no evidence to suggest that beetles are able to control pest insects. Although microsporidia have been reported occasionally from field-collected *Hippodamia convergens*, there is no previous information regarding the prevalence of microsporidia (or other organisms) in lady beetles. In this study, convergent lady beetles were obtained from three commercial insectaries from July to December 2004. Upon receipt, individuals were examined for *microsporidia*, as well as gregarines, fungi and parasitoids. Trials were used to determine performance on arrival and tissue sections were used to describe tissue pathology.

Contributed paper. Monday, 5:20. 42

**Do Johenrea locustae and Paranoosema locustae represent two different developmental sequences of the same species?**

Yuliya Y. Sokolova 1,2, Carlos E. Lange 1, Yuriy S. Tokarev 4 and James J. Fuxa 1

1Department of Entomology, Louisiana State University AgCenter, Baton Rouge, Louisiana 70803, USA, 3Institute of Cytology, Russian Academy of Sciences, St. Petersburg, 194064, Russia, 4Institute for Plant Protection, Russian Academy of Agricultural Sciences, St. Petersburg, 899620, Russia

*Locusia migratoria* (Acrithidae: Oedipodinae) has a wide distribution and is a major agricultural pest in areas of Africa, Asia and Australia. Two subspecies, *L. m. migratorioides* and *L. m. capitata*, are type hosts of two microsporidia, *Paranoosema locustae* and *Johenrea locustae*, respectively. Adipose tissue is the main site of infection of both pathogens. Morphologically, *Paranoosema* and *Johenrea* are remarkably different. *Paranoosema* is diplokaryotic, apansporoblastic, and dispersoblastic, and causes a diffuse infection. *Johenrea* is haplokaryotic, forms sporophorous vesicles, has larger spores, and develops xenomas. *Johenrea* spores isolated from *L. m. capitata* were used to infect an experimental host, the oedipodine *Trimerotropis pallidipennis*. Development and fine morphology of the pathogen, as well as its pathogenesis, were not typical of *Johenrea* but resembled those of *Paranoosema*. Infection of *L. m. migratorioides* with spores produced in *T. pallidipennis* resulted in partial reproduction of “*Johenrea*” characters, but also the formation of “*Paranoosema*-like” spores. Isolation of DNA from *J. locustae*-infected *L. m. capitata* and *T. pallidipennis* with subsequent PCR amplification of microsporidian SSU rDNA with V1-530 primers showed 99-100% similarity with the correspondent region (c. 480 bp) of *P. locustae* DNA in Genbank. We speculate that *J. locustae* might represent a sexual phase of *P. locustae*.
Microsporidian parasites infect almost all invertebrate and vertebrate hosts and have significant effects on individual and population fitness. Phylogenetic analysis demonstrates that the phylum is highly divergent and that some lineages show strong associations with host taxa. We have examined the diversity and distribution of parasites in gastropod molluscs to test for host-parasite co-association. Sixteen populations representing ten species of freshwater snails were screened using microsporidian specific small subunit rDNA primers. Four novel microsporidian parasite sequences were detected within populations of three host species from the genera Bulinus, Biomphalaria and Planorbis. Prevalence ranged from 5% to 84%. Phylogenetic analysis of these novel sequences reveals that they group together as a paraphyletic assemblage in the microsporidian tree basal to the two lineages containing the genera Encephalitozoon and Nosema. Preliminary observation of one microsporidian infection, show parasites distributed in all tissue systems of Bulinus globosus. However, infection is most prevalent in the digestive gland while also in the egg sacs, suggesting that the microsporidium is using a mixed strategy of horizontal and vertical transmission in this population.

Microsporidian parasites may implicate a similar function governing sexual development.

Microarray technology, genomics and proteomics in entomopathogen research

The exhaustive data base provided by genomics of entomopathogens allows us to address the expression, interactions, and functions of the many ORFs uncovered. However, this multitude of genes exceeds the capacity for traditional molecular tools such as Northern blotting and protein expression. DNA microarrays provide a high throughput alternative for evaluating simultaneously the transcriptional profiles of all genes within a genome. For example, one DNA microarray hybridization is equivalent to up to 3,000 or more identical Northern blots, each probed with a different DNA fragment and allowing for comparison of global gene expression under different conditions (e.g. infected vs. uninfected cells). DNA microarrays have been applied to studies on single nucleotide changes (SNPs), loss or amplification of DNA, diagnostics, identifying members of consortia and even DNA replication. This workshop introduces the methodology behind DNA microarray printing, sample preparation, hybridization and data analysis. Using insect viruses as examples, we will explore the application of this technology to viral gene expression and their use in research on other entomopathogens.

Proteomics, following on the heels of this technology, allows researchers to track the synthesis of specific proteins under different conditions and to identify protein-protein partners in a complex milieu of many proteins.

Analysis of Gregarines niphandrosides mitochondria

Gregarines are understudied unicellular parasites of invertebrates. They are members of the phylum Apicomplexa. Many apicomplexans possess two types of extranuclear DNA, mitochondrial and plastid. The plastid genome is small, only 35kb. The apicomplexan mitochondrial genome is the smallest known mitochondrial genome, 6kb. It encodes two proteins: cytochrome C and cytochrome B and small fragments of rRNA. One group of apicomplexans, Cryptosporidium, lacks both the plastid and mitochondrial genomes. Phylogenetic analysis indicates that gregarines and Cryptosporidium are closely related. This relationship raises the question, “Do gregarines possess extranuclear DNA?” Our study investigates the presence and identity of extranuclear DNA in gregarines. We have identified extranuclear DNA in G. niphandrosides trophozoites by 4',6-Diamidino-2-phenylindole (DAPI) staining. We have not found any evidence of a plastid genome within gregarines. We have retrieved a partial sequence of the mitochondrial cytochrome C gene from Gregarina niphandrosides through PCR. Phylogenetic analysis indicates this sequence is a member of the apicomplexan mitochondrial cytochrome C gene family. Southern blot analysis has demonstrated that this gene resides on a fragment of DNA approximately 40kb in size. We are studying the cellular location of this mitochondrial cytochrome C gene in Gregarina niphandrosides.

Microsporidian parasites in freshwater snails

H. Elizabeth McEvoy*1, A.M. Dunn1, R.S. Terry1, D. Rollinson1, D.T.J. Littlewood2 and J. Smith1

1School of Biology, University of Leeds, UK, 2The Natural History Museum, London, UK

Microsporidian parasites infect almost all invertebrate and vertebrate hosts and have significant effects on individual and population fitness. Phylogenetic analysis demonstrates that the phylum is highly divergent and that some lineages show strong associations with host taxa. We have examined the diversity and distribution of parasites in gastropod molluscs to test for host-parasite co-association. Sixteen populations representing ten species of freshwater snails were screened using microsporidian specific small subunit rDNA primers. Four novel microsporidian parasite sequences were detected within populations of three host species from the genera Bulinus, Biomphalaria and Planorbis. Prevalence ranged from 5% to 84%. Phylogenetic analysis of these novel sequences reveals that they group together as a paraphyletic assemblage in the microsporidian tree basal to the two lineages containing the genera Encephalitozoon and Nosema. Preliminary observation of one microsporidian infection, show parasites distributed in all tissue systems of Bulinus globosus. However, infection is most prevalent in the digestive gland while also in the egg sacs, suggesting that the microsporidium is using a mixed strategy of horizontal and vertical transmission in this population.

Parasites were selected for resistance to pyrimethamine and infection, show parasites distributed in all tissue systems of Bulinus globosus. However, infection is most prevalent in the digestive gland while also in the egg sacs, suggesting that the microsporidium is using a mixed strategy of horizontal and vertical transmission in this population.

Microarray technology, genomics and proteomics in entomopathogen research

Microarray technology, genomics and proteomics in entomopathogen research

Peter J Krell1, David A Theilmann*, Luke Masson, Manuella van Munster, Dan-Hui Yang, Ilse Huijskens and Martin A Erlandson

1Dept. of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada, 2Agriculture and Agri-Food Canada, Pacific Agri-Food Centre, Summerland, BC, Canada, 3Biotechnology Research Institute, NRC, Montreal, QC, Canada, 4Faculty of Agricultural Sciences, University of British Columbia, Vancouver, BC, Canada, and 5Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada

The exhaustive data base provided by genomics of entomopathogens allows us to address the expression, interactions, and functions of the many ORFs uncovered. However, this multitude of genes exceeds the capacity for traditional molecular tools such as Northern blotting and protein expression. DNA microarrays provide a high throughput alternative for evaluating simultaneously the transcriptional profiles of all genes within a genome. For example, one DNA microarray hybridization is equivalent to up to 3,000 or more identical Northern blots, each probed with a different DNA fragment and allowing for comparison of global gene expression under different conditions (e.g. infected vs. uninfected cells). DNA microarrays have been applied to studies on single nucleotide changes (SNPs), loss or amplification of DNA, diagnostics, identifying members of consortia and even DNA replication. This workshop introduces the methodology behind DNA microarray printing, sample preparation, hybridization and data analysis. Using insect viruses as examples, we will explore the application of this technology to viral gene expression and their use in research on other entomopathogens.

Proteomics, following on the heels of this technology, allows researchers to track the synthesis of specific proteins under different conditions and to identify protein-protein partners in a complex milieu of many proteins.
Trichoctosporea inhabiting hydrobionts. Spores of Study of life cycles and transmission of parasites included larvae of Microsporidium crustaceans (16 species of Cyclopoidea, 3 species of Calanoida) and used for oral infections of naupliar, copepodite stages and adult individuals of crustaceans in laboratory conditions (room temperature from 14 to 25°C) were conducted with spring, summer, autumn and winter generations lower crustaceans and insects.

Study of life cycles and transmission of parasites included experiments cross-infections between mosquito larvae and co-inhabiting hydrobionts. Spores of Parathelohania, Amblyospora and Trichoctosporea species from Anoph eles and Aedes mosquitoes were used for per oral infections of nauplii, copepodite stages and adult crustaceans (16 species of Cyclopoidea, 3 species of Calanoida) and insects (larvae of a dragon-fly and a may-fly). In the same water-bodies we detected microsporidial infections (Microsporidium sp.) of Daphnia and Cyclops species. We did not identified this parasites, but used the collected spores for per oral infection of laboratory culture larvae of Anopheles atroparvus. Spores were used in different ways (fresh-extracted and after storage from 1 month to 4 years in refrigirator or at room temperature (from 0 to 25°C). Experiments were conducted with spring, summer, autumn and winter generations of crustaceans in laboratory conditions (room temperature from 14 to 28°C) for periods from 14 days to till larvae perishes. In all instances infections were not detected. As a result, we did not registered peroral transmission of microsporidians of octopocerc genera from blood-sucking mosquitoes to the same or another species of mosquitoes, lower crustaceans and insects.

Epizootiology of a microsporidium in a blood-sucking mosquito population of Siberia, Russia

Tamara Pankova1 and Anastasia Simakova2

1Chair of Invertebrate Zoology, Tomsk State University, Tomsk, Russia, 2Laboratory of Electron Microscopy, Branch of Federal State Unitary Enterprise of Ministry of Public Health of Russian Federation, Tomsk, Russia.

Seasonal prevalence of microsporidian infections in Aedes mosquitoes reveals, that larvae with clear symptoms of infection detected from first registration of 3rd - 4th instar larvae and to the end of larvae development period, when mass pupation and imago hatching occur. As microsporidian infection resulted in retardation of development, the proportion of infected larvae increases while total number of 4th instar larvae decreases. Anopheles mosquitoes have 2 or 3 generations and infected larvae were registered in the first generation. The number of infection peaks coincides with the number of maximums of host population density. And the highest rates of infection were registered in the end of summer period because of maximums of host population density. And the highest rates of infections independently of numbers of host species. In Anopheles populations with Parathelohania sp. infection rates varied from 0.1 to 1.6%, in Aedes populations with Amblyospora infections from 0.1 to 6.5%.

Exploring horizontal field transmission of Microsporidia

Vincent D'Amico1, Leellen Soltis2, Milan Zubrik3, Michael McManus4, and Gernot Hoch5

1USDA-FS, University of Delaware, Newark DE, USA, 2Illinois Natural History Survey, Champaign IL, USA, 3Forest Research Institute, Zvolen Slovakia, 4USDA-FS, Hamden CT, USA and 5University of Natural Resources and Applied Life Science, Vienna Austria

How does transmission of microsporidia occur in the field? The known portion of the life cycle of microsporidia in the genus Nosema (for example) is as follows: after ingestion by the host, infective spores germinate in the midgut lumen of the host larva. The polar filament pierces the midgut epithelial and muscle cells of the host and the sporoplasm enters the cell. After a cycle of vegetative reproduction, primary spores are produced, which immediately extrude their polar filaments. Filaments allow spores to infect target tissues such as salivary glands, fat body, and Malpighian tubules. The release of spores from some of these targeted tissues into the environment, in silk and/or feces, is a key step of transmission, but details of this process are poorly understood. For the past two years (2004 and 2005) we have attempted to quantify transmission of a microsporidian infection in forest lepidopteran larvae: as far as we are aware this is the first time such an experiment has been performed under semi-field conditions. The microsporidium we used for this work was a Nosema sp., isolated from a L. dispar population near Levishite, Bulgaria. L. dispar larvae (1st day in 3rd instar) were inoculated and marked by clipping the first left proleg. A study plot was established on a young oak plantation with trees of about 2 to 3 m height near Cifare, Slovakia. Cages (1x1x2 m) were installed around these trees, and infected and uninfected L. dispar were placed in the cages. Larvae were removed after 21 d of exposure. Initially-infected and test larvae were separated according to proleg markings. Initially-infected larvae were frozen and diagnosed for microsporidia under phase contrast microscopy (400X). Susceptible larvae were reared individually for another 11 d before microscopic examination.
Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts

Helen Roy\textsuperscript{1}, Don Steinkraus\textsuperscript{2}, Jorgen Eilenberg\textsuperscript{3}, Ann Hajek\textsuperscript{4} and Judith Pell\textsuperscript{5}

\textsuperscript{1}Department of Life Sciences, Anglia Polytechnic University, East Road, Cambridge, CB1 1PT, UK, \textsuperscript{2}Department of Entomology, 319 AGRI, University of Arkansas, Fayetteville, AR 72701, USA, \textsuperscript{3}Department of Ecology, The Royal Veterinary and Agricultural University, 1871 Frb. C., Denmark, \textsuperscript{4}Department of Entomology, Cornell University, 6126 Comstock Hall, Ithaca, New York 14853-0901, USA, \textsuperscript{5}Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Invertebrate pathogens and their hosts are taxonomically diverse. Despite this, there is one unifying concept relevant to all such parasitic associations: both the pathogen and host will adapt to maximize their own reproductive output and ultimate fitness. The strategies adopted by pathogens and hosts to achieve this goal are almost as diverse as the organisms themselves but studies examining such relationships have traditionally concentrated on aspects of host physiology. Here we report on a review of examples of host-altered behavior and consider these within a broader ecological and evolutionary context. Research on pathogen-induced/host-mediated behavioral changes demonstrates the range of altered behaviors exhibited by invertebrates including behaviorally induced fever, elevation seeking, reduced or increased activity, reduced response to semiochemicals, and changes in reproductive behavior. These interactions are sometimes quite bizarre, intricate, and of great scientific interest.
highlights data that are not routinely collected but could be and that would improve the quality of model predictions. I will also indicate where the development of in theory is currently hampered for want of appropriate data and to which invertebrate pathologists could make a valuable contribution.

Symposium. Tuesday, 8:24. 56
Factors affecting transmission of fungal pathogens of aphids
Don Steinkraus

Department of Entomology, University of Arkansas, Fayetteville, AR 72701, USA

In terms of biological control, fungal pathogens are the most important microbial agents for control of pest aphids. There are many species of fungi that attack aphids and epizoitcs are common. Much has been learned about the factors that affect transmission of aphid pathogens such as: aerial movement of conidia, relative humidity, sunlight, host density, resting spore biology, movement of infected alatae, behavior of infected aphids, and host range. In spite of this many questions remain and manipulating fungi to produce epizootics in aphids remains a difficult feat. Examples of what is currently known about fungal pathogens of Aphis gossypii, Aphis glycines and other species will be discussed, particularly the pathogen Neocygistes fresei.

Symposium. Tuesday, 8:48. 57
Consideration of vertically transmitted microsporidia for biological control
Leelien F. Soltner

Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820, USA

Vertical transmission is common for pathogens of relatively low virulence because successful larval development, adult emergence, mating and reproduction of the infected host must occur after an infection is acquired, usually during the larval stages. Most microsporidia that are vertically transmitted are also horizontally transmitted, whether in a single host species or via an intermediate or alternate host. This would appear to favor the persistence of microsporidia released as classical biological control agents against their natural hosts. From a different perspective, successful transovarial transmission requires an explicit interaction between a pathogen and its host, and probably limits the ability of microsporidia to host switch. Laboratory studies of European corn borer, Ostrinia nubilalis larvae challenged by Nosema spp. and Fairimorpha spp. isolated from other stem-boring and row crop hosts showed that transmission, both horizontal and vertical, is a barrier to successful invasion of nontarget species. Vertical transmission is probably more stringent than horizontal transmission. Transmission experiments, therefore, may provide a more refined laboratory test of host specificity for microsporidia that are orally infective to nontarget hosts.

Symposium. Tuesday, 9:12. 58
Transmission of viruses to mosquito larvae mediated by divalent cations
James J. Becnel and Susan White

U. S. Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL 32608, USA

The most common occluded viruses of mosquitoes are baculoviruses (nucleopolyhedroviruses, NPV) and cytopoviruses (CPV). Mosquito NPV’s have a circular, double-stranded DNA genome packaged into rod-shaped enveloped capsids embedded in a protein matrix. Mosquito cytopoviruses are RNA viruses with a 10 segmented genome packaged into an icosahedral virion. Replication, assembly and occlusion of CPV’s occurs in the cytoplasm of midgut epithelial cells. Historically, both mosquito NPV’s and CPV’s have been difficult to transmit to the larval host. Studies on an NPV from Culex nigripalpus (CuniNPV) revealed that transmission is mediated by divalent cations: magnesium is essential, whereas the presence of calcium inhibits the activity of magnesium. Transmission of a second baculovirus (UrsaNPV) is also enhanced by the presence of magnesium. Transmission studies with a CPV from Uranotaenia sapphirina (UsCPV) have shown a 30 fold increase in infectivity when magnesium is present. Calcium inhibits the activity of magnesium to facilitate transmission of UsCPV. The role these divalent ions play in either enhancing or inhibiting transmission is unknown. It is interesting that two distantly related virus groups have similar transmission requirements suggesting that the divalent ions interact with components of the mosquito midgut rather than directly with virions of the virus.

Symposium. Tuesday, 9:36. 59
Effect of mono- and poly-gyne social forms on transmission and spread of microsporidia in fire ant populations
David Oi

USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, 32608, USA

Thelohania solenopsae, a pathogen of red imported fire ants, Solenopsis invicta, can be transmitted by introducing infected brood into a colony. The social form of the ant, that is, monogyne (single queen per colony) or polygyny (multiple queens per colony) are associated with different behaviors, such as territoriality, that affect the degree of intercolony brood transfer. T. solenopsae was found only in polygynous colonies (83%) in Florida. Non-synchronous infections of queens and transovarial transmission favor the persistence and probability of detecting infections in polygynous colonies. However, queens or alates with the monogyne genotype can be infected and infections in monogynic field colonies of S. invicta have been reported from Louisiana and Argentina. Alate queens with the monogyne genotype have a greater dispersal capability than polygynous alates and could potentially facilitate the spread of the pathogen. Demise of infected monogynous colonies can be twice as fast as in polygynous colonies and favors the pathogen’s persistence in polygynous fire ant populations. The social form of the fire ant reflects different physiological and behavioral aspects of the queen and colony that will impact T. solenopsae spread and ultimate usefulness for biological control.

Symposium. Tuesday, 8:00. 60
Emerging genomics of fungal entomopathogens

Symposium. Tuesday, 8:00. 60
Generation of a robust EST dataset for the entomopathogenic fungus Beauveria bassiana
Eun-Min Cho and Nemat O.Keyhani

University of Florida, Microbiology and Cell Science, Bldg 981, Museum Rd. Gainesville, FL 32611, USA

Beauveria (Cordyceps) bassiana is a broad host range entomopathogenic fungus under intensive study as an arthropod biocontrol agent. Strains of B. bassiana have been selected for directed virulence towards insects and other arthropods that act as agricultural pests, disease vectors, ecologically hazardous, invasive pests, and even household nuisance pests. B. bassiana produces at least three distinct single cell propagules including aerial conidia, in vitro vegetative cells termed blastospores, and microcyle conidia that can be isolated from agar plates, rich broth liquid cultures, and under conditions of nutrient limitation in submerged cultures, respectively. cDNA libraries were constructed from each B. bassiana cell type and a robust expressed sequence tagged (EST) dataset was generated. Additional cDNA libraries from cells sporulating on chitin and producing the secondary metabolite oosporein also contributed to yield a diverse array of transcripts. Approximately 2,000 clones from each library were sequenced and a unique sequence set was constructed. Comparative analysis of the expressed transcripts in each library indicated significant differences in gene expression pattern between the cell types in several broad categories including cell wall biosynthesis, secondary metabolism, and the production of proteases.
Let us assume that a complete genomic sequence of *Beauveria bassiana* or *Metarhizium anisopliae* will be available in the near future. Which fungus will or should be the sequenced first? Which isolates should be chosen? Once these fungi are sequenced, some major questions need to be asked: Which fungi should be next and why? Which NONentomopathogenic fungi need sequencing to provide appropriate standards to evaluate the entomopathogen’s genes? Innumerable such specific questions arise. And questions must also be raised about what will be done with the information gained from such sequences. Other speakers in this symposium address some of these questions, but it is also time to ask some very big questions that might help to unlock greater understandings of the systematics, phylogeny, ecology, and overall organismal and population population of these fungi. Now that the complete life histories of *Beauveria, Metarhizium,* and other clavicipitaceous entomopathogens are better understood, it will be time to push boldly towards an integration of our understandings of these fungi, their roles in the environment, and how they operate throughout their life history in a manner wholly unanticipated only a few short years ago.

**NEMATODES AND SYMBIOTIC BACTERIA**

Contributed paper. Tuesday, 8:00. 64

**Insecticidal toxins from *Photorhabdus* bacteria**

Richard ffrench-Constant, Nicholas Waterfield, Andrea Dowling, Guowei Yang

Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK

We will present an update of our work on three different insecticidal toxins produced by the insect pathogenic bacterium *Photorhabdus*. We will discuss recent work on the orally active toxin complexes (TeCs) in the light of recent findings that three genes are necessary for full toxicity: an A toxin and a B-C potentiator pair. We will also provide an update on the mode of action of the major injectably active apoptosis-inducing toxin Makes caterpillars floppy (Mcf). Finally, we will discuss recent work on the novel binary toxin termed *Photorhabdus* insect related protein (PirAB) which shows similarity both to Bt delta-endotoxins and also to a developmentally regulated protein in beetles, causing it to be confusingly named a Juvenile Hormone Esterase (JHE)-like protein. We will provide evidence that PirAB lack JHE activity but represents yet another insecticidal toxin from this bacterium- this time with potent oral activity against mosquito larvae.

Contributed paper. Tuesday, 8:15. 65

**Mixing and matching of toxin complex proteins**

Timothy Hey, Scott Bevan, Amanda Schleper, Patricia Birkhold, Stephanie Burton, Tom Meade, Don Merlo, Joel Sheets, Robin Thompson and Haley Moon

Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268, USA

Bacteria in the genera *Photorhabdus* and *Xenorhabdus* produce several classes of Toxin Complex proteins. The Class A proteins (~280 kDa) possess insecticidal activity. The Class B (170 kDa) and Class C proteins (~110 kDa) possess no apparent insecticidal activity. Several laboratories have demonstrated that potent insecticidal activity requires all three classes of protein (A, B and C) with the Class B and Class C proteins potentiating the activity of the Class A proteins by as much as 1000 fold. We tested the insecticidal activities of fifteen toxin complex proteins alone, and in combination with one another. Several examples from Class A, B and C were studied. The proteins were derived from four species (three genera; *Photorhabdus*, *Xenorhabdus* (Gram negative) and *Paenibacillus* (Gram positive)). Our data show that Toxin Complex proteins from widely divergent sources may be mixed and matched to provide potent insecticidal activity. It appears that the Class A proteins are responsible for determining activity spectrum and the potentiators (Class B + C) the
Novel toxin complex constructions

Timothy Hey, Charles Cai, Aaron Woosley, Stephanie Burton, Joel Sheets, Brian Waldman, Haley Moon, Tom Meade and Don Merlo
Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268, USA

The toxin complex proteins from *Photonhagus* and *Xenorhabdus* represent a new broad class of highly potent insect control agents. The active complex consists of a tetramer of a Class A protein (~280 kDa/subunit) a single Class B protein (~170 kDa) and a single Class C protein (~110 kDa). Toxin Complex has been considered as a possible candidate for use in transgenic plants, especially since Bt resistant insects are susceptible to Toxin Complex. We have designed novel Toxin Complex gene constructions to increase plant transformation efficiency and to provide coordinated expression of three genes in plants.

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

The characterisation of the structure of *Xenorhabdus* insecticidal toxin component XptA1

Sarah C. Lee¹, Svetla McPhie², Alison Rodger¹, David I. Roper¹,
Janey Henderson¹, Martin Sergeant¹ and J. Alan W. Morgan¹
1Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF, UK, 2Department of Biological Sciences, University of Warwick, Gibbets Hill, Coventry, UK, 3Department of Chemistry University of Warwick, Gibbets Hill, Coventry, UK, 4University of Nottingham, Nottingham, UK, 5School of Biological Sciences, Coventry University, UK

There is an interest in new and novel protein toxins, with activity against a wide range of pests, particularly new toxins with different modes of action to the current *B. thuringiensis* toxins. A series of highly active insecticidal protein toxins have been described from bacteria found associated with insect parasitic nematodes (*Xenorhabdus* species). From one bacterial strain (*Xenorhabdus nematophila* pMFL019) toxins that are able to kill the insects *Piers brassicae*, *Phadella xylostella*, *Heliothis variscens* and *H. zea* have been identified. Three classes of genes have been shown to be involved in insecticidal activity. These have been named *xptA*, *xptB* and *xptC*. XptA1 was expressed in *E. coli* and purified. Circular dichroism analysis showed that the protein contained a high proportion of α-helices as its predominant secondary structural feature. Its monomeric unit of 280,000 Da formed a tetramer, as determined using Dynamic light scattering and Analytical Ultracentrifugation. The purified sample was shown to be monodisperse, with a predicted spherical diameter of 20 nm and molecular weight of 1.2MDa. Further studies using Transmission Electron Microscopy (TEM) and negatively stained protein also revealed that the protein was a tetramer. Using Electron Micrograph analysis program (EMAN), single particle reconstruction of a 10,000 particle data set was carried out giving a 3D model and electron density contour map of the XptA1 protein to a resolution of 25 Å. This represents the first structural characterisation of this large bacterial insecticidal toxin. Because of the Insecticidal nature of these proteins, and their possible use in the control of invertebrate pests, this work is key to the elucidation of the mode of action of these proteins.

**STU** Contributed paper. Tuesday, 9:00: 68

The hemolysin alpha-xenorhabdolysin secreted by pathogenic enterobacteria belongs to a new family of cytotoxins and triggers apotosis

Fabienne Vigneux¹, Alain Givaudan¹, Pierre Alain Girard², Carlos Ribeiro¹, Stephen Baghdiguian¹ and Michel Breblié¹
1Laboratoire d’Ecologie Microbienne des Insectes Interactions Hôtes-Pathogènes UMR 1133 INRA-Université de Montpellier II, 34090 Montpellier, France, 2Institut des Sciences de l’Evolution, UMR 5554, Université de Montpellier II, 34090 Montpellier, France

*Xenorhabdus nematophila* is a Gram negative bacterium belonging to the family of Enterobacteriaceae, symbiotically associated with the nematode *Steinernema carpocapsae* (*Steinernematidae*). This entomopathogenic couple kills insects belonging to numerous orders after a potent depression of the immune system. *X. nematophila* is highly pathogen by itself and is able to escape defence reactions and especially phagocytosis. The α-Xenorhabdolysin (αX), a toxin with cytolytic activity on insect hemocytes (immunocytes) and haemolytic activity on sheep red blood cells (SRBC), was purified from *X. nematophila*. Granulocytes, which are the functional equivalent of vertebrate macrophages, were the most susceptible hemocytes to αX, which elicits apoptosis in these insect immunocytes. Peptide sequencing of this cytotoxin allows us to clone the gene coding for this protein. Its expression in transfected *E. coli* allows the production of large amounts of this cytotoxin/hemolysin with exactly the same activity on insect hemocytes and SRBC than the lysin purified from *X. nematophila* broth growth. According to nucleotide and amino acid sequences, αX cannot be classified in an already known family of cytotoxins but belongs to a new family. This work gives new insights in the understanding of the bacteria-host relationships.

**STU** Contributed paper. Tuesday, 9:15: 69

Effect of harvest time and culture conditions on the morphology and ultrastructure of the bacterial receptive in *Steinernema carpocapsae* (Nematoda: Steinernematidae)

S. Patricia Stock¹ and Yolanda Vega²
1 Department Plant Sciences-Department of Entomology, University of Arizona, Tucson, AZ 85721, USA, 2 Department Plant Sciences, University of Arizona, Tucson, AZ 85721, USA

One of the central facets of the *Steinernema-Xenorhabdus* symbiosis is the third-stage juvenile nematode (IJ). The IJ is colonized by a monoculture of *Xenorhabdus* bacteria at a discrete structure located in the anterior portion of the intestine known as the “bacterial vesicle” or “intestinal vesicle”. Yet, the physiological and developmental processes by which any steinernematid IJ is colonized by its bacterial symbiont are yet not well understood. It has been suggested that age of the nematode population and culture conditions influence the degree of colonization of the intestinal vesicle. In turn, the level of bacterial colonization may affect the morphology and ultrastructure of the vesicle and connecting structures. We evaluated the conditions that affect morphological and ultrastructural changes of the bacterial receptive of *S. carpocapsae*. Light (DIC) and Transmission Electron Microscopy (TEM) were considered to evaluate variation in size and shape of the vesicle of *S. carpocapsae* considering IJ populations of different age and from different culture conditions. Preliminary data indicates there is a negative correlation between IJ body size and vesicle size with harvest time, with both smaller IJ body and vesicle size as the harvest time increases. However, when compared with freshly emerged IJs (day 0) differences in size of nematodes and bacterial vesicle (width) were only significantly different (P < 0.05) for IJs harvested after 28 days. No significant differences were recorded for the length of the bacterial vesicle between days 7 to 21. Size variation was also observed in IJs grown at different culture conditions (i.e. *in vivo vs in vitro*), with the IJ body and vesicle size being smaller in *in vitro*-reared nematodes. We speculate changes in the vesicle width are related to a decrease of bacterial cfu. Levels of bacterial colonization and viability might be greater in the nematodes grown in vivo. A progressive decline in bacterial colonization has also been observed as storage time increases. All these factors might be related to a reduction (constriction) of the body wall of the bacterial receptive, therefore reducing its size as bacteria colonies decrease.

**STU** Contributed paper. Tuesday, 9:30: 70

Genetic and molecular analysis of infective juvenile longevity in the entomopathogenic nematode *Heterorhabditis bacteriophora*

Sukhinder K. Sandhu and Parwinder S. Grewal
Department of Entomology, The Ohio State University, OARDC, Wooster, OH 44691, USA

Poor storage stability is a major obstacle to the expansion of entomopathogenic nematode use in biological control. One of the
main reasons being the short longevity of the nematode infective juveniles (IJs). We established inbred lines in *Heterorhabditis bacteriophora* to determine genetic variability in longevity of the IJs. The IJ longevity assessed, as LT₉₀ in weeks was significantly different among the inbred lines and varied between 16 to 20 weeks. In a separate study of the cDNA library of the *H. bacteriophora* IJs, we found homologs of *Caenorhabditis elegans* aging genes and selected four candidate genes to determine the differences in their expression in a long- and a short-lived inbred line. Two genes AKT/PKB kinases, akt-1 and pdk-1, are members of the insulin-like signaling pathway which regulates growth, metabolism and longevity and the other two, superoxide dismutase, sod-4 and heat shock protein, hsp-4, are stress resistance genes. We hypothesized that akt-1 and pdk-1 are upregulated in the short-lived inbred line and downregulated in the long-lived line and sod-4 and hsp-4 are upregulated in long-lived and downregulated in short-lived line based on their role in *C. elegans*. Real-time RT-PCR approach is followed for quantification of the respective candidate genes.

Contributed paper. Tuesday, 9:45.

**Characterization of surface coat proteins from *Steinernema glaseri* that suppress immune responses in *Oriental beetles larvae***

Xinyi Li¹, Richard S. Cowles², Elizabeth Cowles³, Randy Gaugler⁴, A. Daniel Jones⁵ and Diana L. Cox-Foster⁶

¹Department of Entomology, The Pennsylvania State University, University Park, PA 16802, ²Valley Laboratory, The Connecticut Agricultural Experiment Station, Windsor, CT 06095, ³Department of Biology, Eastern Connecticut State University, Willimantic, Connecticut 06226, ⁴Department of Entomology, The Rutgers University, New Brunswick, NJ 08901, ⁵Department of Chemistry, The Pennsylvania State University, University Park, PA 16802, USA

Infective juveniles (IJ) of *entomopathogenic nematodes* (EPNs) penetrate insect hosts and release symbiotic bacteria that kill the insect hosts. Insect hosts defend against EPNs by a rapid cellular immune response that results in encapsulation and melanization that kills EPNs. The nematodes have to overcome the innate immunity of the hosts to survive and reproduce. Therefore, the release of symbiotic bacteria has to occur before the intensive host immune responses. Surface coat proteins (SCPs) of EPNs are suggested to play a role in suppression/evasion of host immune responses. We showed that different species and strains EPNs have different surface coat protein profiles. We isolated and characterized surface coat proteins from *Steinernema glaseri* NC strain. These SCPs suppressed immune responses of the *Oriental beetle* larvae, a susceptible host for *S. glaseri*, thus protecting *Heterorhabditis bacteriophora* from being killed in the same host, as it normally would be. The immune suppression is dose dependent. Also, multiple injections of the SCPs protected *H. bacteriophora* better in the *oriental beetle* larvae. In nonadenated state, two isolated proteins in the SCPs of *S. glaseri* each convey this immuno-suppressive effect. The two proteins are composed of smaller proteins when separated on two dimensional PAGE. The sequences and characterization of the proteins were also investigated.

**CONTRIBUTED PAPERS. Tuesday, 8:00-10:00**

**VIRUSES-2**

Contributed paper. Tuesday, 8:00.

**A cell culture system and infectious clone for the study of *Rhopalosiphum padi* virus (Diciistroviridae)**

Sandhya Boyapalle¹, Randy Beckert², W. Allen Miller², Bryony C. Bonning³

¹Departments of Entomology and ²Plant Pathology, Iowa State University, Ames, IA, USA

*Rhopalosiphum padi* virus (RhPV) is an icosahedral aphid virus with a 10 kb positive-sense RNA genome. We screened lepidopteran, dipteran and homopteran cell lines for susceptibility to RhPV following RNA transfection and observed cytopathic effects in homopteran cell lines derived from the glassy winged sharp shooter, *Homalodisa coagulata*, and the corn leaf hopper, *Dalbulus maidis*. Infection, viral replication and production of virions was confirmed by northern blot hybridization, RT-PCR, Western blot analysis and immunoelectron microscopy. Full-length cDNA clones of RhPV were synthesized. RNA transcripts produced from one of the clones were infectious following transfection of the susceptible cell lines. Infection was confirmed by CPE and immunoelectron microscopy. Virions were purified from infected cells and fed to bird cherry-oat aphids, *Rhopalosiphum padi*. Aphids tested positive for infection by the RhPV clone by RT-PCR, Western blot analysis and immunolocalization by light microscopy, two weeks after acquisition in three out of three replicates.

Contributed paper. Tuesday, 8:15.

**Baculovirus expression of *Rhopalosiphum padi* virus (Diciistroviridae)**

Sandhya Boyapalle¹, Randy Beckert², W. Allen Miller², Bryony C. Bonning³

¹Departments of Entomology and ²Plant Pathology, Iowa State University, Ames, IA, USA

A full length cDNA clone of the *Rhopalosiphum padi* virus (RhPV) genome was inserted into the genome of *Autographa californica* nucleopolyhedrovirus to create the recombinant baculovirus AcRPV6. Expression of the RhPV genome in *S. frugiperda* cells resulted in formation of RhPV virus-like particles (VLPs) whose capsids are structurally and immunologically indistinguishable from the native virions. The presence of genomic RhPV RNA in recombinant baculovirus infected cells and in VLPs was confirmed by RT-PCR. Assembly of RhPV VLPs in the nucleus of baculovirus infected cells suggests that in *S. frugiperda* cells (i) both the 5' and IGR IRES of RhPV are active, (ii) the virus encoded protease is functional for processing of RhPV polyproteins, and (iii) replication of RhPV is not required for encapsidation of RNA. For analysis of the infectivity of baculovirus expressed RhPV6, virions purified from baculovirus-infected *S. frugiperda* cells were fed to the bird cherry-oat aphid, *Rhopalosiphum padi*. Aphids were tested for infection by the baculovirus-produced RhPV clone by RT-PCR and western blot analysis, four weeks after acquisition. Baculovirus expression of RhPV in lepidopteran cell lines that do not support replication of RhPV provides a potential alternative approach for *in vitro* production of clones of this virus.

**Characterisation of a new virus isolated from the rosy apple aphid, *Dysaphis plantaginea***

Neil Naish, Eugene Ryabov and Doreen Winstanley

Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK

The role of aphids as vectors of plant viruses and their relationship with the host plant is well documented. However, the potential of plants to act as reservoirs for aphid pathogenic viruses and interactions of aphid pathogenic viruses within their complex lifecycles is unknown. Rosy apple aphid (RAA) (*Dysaphis plantaginea*) is the most destructive aphid on apples in South-East England. During the hot summer months, the virginsparae feed on plantain and the morphology of the aphid differs depending on the host plant. The fall and spring winged adults migrate to and from the apple and the aphid over-winters as ova on apple trees. In 2003, a virus was isolated from a laboratory culture of RAA, maintained at Warwick HRI and is closely related to *Acyrthosiphon pisum* virus (APV). The complete virus genome sequence has been determined and genetic analysis is in progress. The RAAV genome is 9971 nucleotides in length, excluding the 3' end poly(A) tail, and contains two large open reading frames, encoding proteins of 296 KDa and 63 KDa. Results on the biological characterisation of the RAA virus, its pathogenicity to RAA and other aphid species and its relationship with its main plant hosts will be reported.
Comparative viral RNA loads in deformed wing virus infected *Apis mellifera* L. and its ectoparasite *Varroa destructor*

Diana Tentecheva, Laurent Gauthier, Benjamin Dainat, François Cousserans, Marc Edouard Colin and Max Bergoin

Laboratoire de Pathologie Comparée des Invertébrés EPH, UMR1231 Biologie Intégrative et Virologie des Insectes, Université Montpellier II, 34095 Montpellier, France

A two step quantitative RT-PCR assay was validated to monitor the deformed wing virus (DWV) RNA loads in *Apis mellifera* L. and *Varroa destructor*. A couple of primers hybridising in a conserved domain of the putative DWV RNA polymerase gene region was designed. These primers amplified a 69 nucleotide fragment which was quantified using the SYBR-green chemistry. The experimental validation of the method showed that the RNA extraction step was responsible for the greatest variability in the results while assays repeated on different PCR plates were shown reproducible. The quantitative RT-PCR analysis on bee workers showed that very large DWV RNA loads can be recorded in bees in absence of clinical signs, validation of the method showed that the RNA extraction step was responsible for the greatest variability in the results while assays repeated on different PCR plates were shown reproducible.

Analysis of the Immediate early *me53* gene from the baculovirus AcMNPV

Jondavid de Jong1, David A. Thielmann2, Basil M. Arié2 and Peter J. Krell1

1Dept. of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada, 2Agriculture and Agri-food Canada, Pacific Agri-food Centre, Summerland, BC, Canada, 3Canadian Forest Service, Great Lakes Forestry Research Centre, Sault Ste. Marie, ON, Canada

Over the past 50 years the eastern spruce budworm, *Choristoneura fumiferana*, has been the most destructive forest insect pest in North America. Management of such pests has relied heavily upon the use of chemical pesticides which tend to harm both non-target and target organisms. Recently, the focus of research has shifted to the use of biological control agents such as the baculovirus *C. fumiferana* multiple nucleopolyhedrovirus (CfMNPV). Baculoviruses encode five major immediate early transcripts corresponding to ie1, ie-0, ie-2, pe38 and me53. Four of these, ie-1, ie-2, ie-0 and pe38 have been studied in detail and have been found to play vital roles in viral infection. In the type virus, *Autographa californica* MNPV, me53 is expressed at high levels from a immediate-early promoter. Although the gene has been defined transcriptionally, no attempt has been made to determine the function of me53. Me53 encodes a putative protein containing a C-4 zinc-finger and is thought to be involved in transcriptional transactivation. We have created a me53-null AcMNPV virus and have compared it to wild-type virus on the basis on infectivity, virus yield and DNA replication. Additionally we have tagged the me53 protein so it can be analyzed in protein studies.

Characterisation of *Cydia pomonella* granulovirus metalloproteinase

E. M. Kemp, S. L. Hilton, and D. Winstanley

Warwick HRI, Wellesbourne, Warwick CV35 9EF, UK

All granuloviruses (GVs) sequenced to date possess a zinc metalloproteinase homolog. Sequence analysis reveals homology to the catalytic domain of the matrixin family, but the GV metalloproteinases (*mp*) do not possess the conserved cysteine-switch region, involved in activation, common to the matrix metalloproteinases. Six of the seven GV metalloproteinases possess a putative signal peptide suggesting they may function extracellularly. In order to investigate the function of the metalloproteinase in GV infection a recombinant *Cydia pomonella* GV (CpGV) lacking mp was produced. Sequence analysis revealed no recognised eukaryotic promoter sequence upstream of mp. A luciferase reporter assay was carried out to determine the temporal class of the mp promoter. Metalloproteinase activity assays were performed in vitro to determine if the signal peptide is functional. Another CpGV protease, v-cath, known to be essential for liquefaction and melanization in AcMNPV, was also deleted from the CpGV genome in order to investigate its function in CpGV. Results of the deletion and promoter studies suggest that mp is a non-essential early gene and is not required for liquefaction.
Symposium. Tuesday, 10:20. **80**

**A polydnavirus paradox: Cophylogeny and mosaic genomes**

James Whitfield  
University of Illinois, USA

The polydnaviruses associated with braconid parasitoid wasps have been shown to be restricted to a large lineage of the wasps, and to be inherited apparently by all individuals of this lineage. Expectations were therefore that the polydnaviruses and wasps would be found to have coevolved phylogenetically since a single original association. The braconids that carry the viruses form a monophyletic group that appears to have arisen about 74 million years ago, based on fossil-calibrated molecular clock estimates. Phylogenetic studies of braconoviruses carried by wasps within the wasp genus *Cotesia* show that the polydnavirus phylogeny, as inferred from the viral gene homologs of EP1 and CrV1, matches that of the wasps, as inferred from DNA sequences from the genes 16S, ND1, 28S and longwave opsin. Attempts to show that the cophylegetic pattern extends throughout the braconvirus-bearing wasp lineage have so far not been successful. The recently fully sequenced genomes of several polydnaviruses now show that the viruses are far more complex and composite in nature than originally realized, with multiple large gene families of different mosaic histories. Thus, progress in determining the early coevolutionary history of the wasps and viruses will depend upon either using phylogenetic network methods to untangle these mosaic histories, or identifying single-copy viral housekeeping genes for analysis. Some promising prospects of both strategies are presented.

Symposium. Tuesday, 10:50. **81**

**Polydnavirus genomics: Form and function of mutualistic insect viruses from parasitic wasps**

Bruce A. Webb  
Department of Entomology, University of Kentucky, Lexington, KY 40546-0091, USA

The polydnaviruses are an unusual group of viruses that exist in obligatory symbiosis with the genomes of parasitic wasps. The genomes of a number of polydnaviruses have recently been sequenced and are found to be largely non-coding, encode gene families and have few genes that are related to genes from other viruses. The polydnavirus genes that can be identified by comparison to database sequences are related to genes from eukaryotic cells. In this presentation, I will summarize the data from the available sequenced genome and compare and contrast the polydnavirus genomes from the different phylogenetic clades. This comparison will identify shared and unique features between and within polydnavirus lineages in hopes of revealing evolutionary processes that have acted on these genomes and thereby develop testable hypotheses that may orient and drive future research in this field.

Symposium. Tuesday, 11:20. **82**

**Inferring evolution through the biology of ascoviruses**

Xiao-Wei Cheng  
Department of Microbiology, Miami University, Oxford, Ohio 45056, USA

Ascoviruses are transmitted to lepidopteran larvae by parasitoids mechanically during oviposition. There are four official ascovirus species accepted by ICTV. The *Diacromus pulchellus ascovirus 4* (DpAV-4), which has a genome of 116 kb, replicates in the female genitalia. The *Spodoptera frugiperda ascovirus 1* (SfAV-1) has a genome of 140 kb, infects only *Spodoptera* spp., and replicates in the fat body tissue. The genomes of *Heliolissis virensis 3* (HvAV-3) and *Trichoplusia ni ascovirus 2* (TnAV-2) are about 180 kb. The two have a similar wider host spectrum, with HvAV-3 replicating in the fat body and TnAV-2 replicating preferentially in the epidermis, tracheal matrix and connective tissues. It is hypothesized that the four ascoviruses evolved according to the genome size, host range, tissue tropism and degree of independence on replication in hymenopteran hosts from DpAV-4 to SfAV-1 to HvAV-3 to TnAV-2. Such evolution might have occurred by acquiring genes horizontally from hosts thereby increasing genome size, expanding host range and widening tissue tropism. In addition, iridoviruses, ascoviruses and poxviruses are all cytoplasmic viruses. Evolution might have occurred from a more nuclear replication dependent iridovirus to a complete nuclear replication independent entomopoxivirus in cytoplasm with ascovirus as a link.
Genomics approaches to insect-pathogen relationships in the spruce budworm, *Choristoneura fumiferana*  
Qili Feng1, Tim Ladd1, Sichun Zheng1,2, Lan Li1, Dayu Zhang1,2, Debra Buhlers1, Peter J. Kreil2, Basin M. Arit2, Arthur Retnakaran1

1Great Lakes Forestry Centre, Canadian Forest Service, 1219 Queen Street East, Sault Ste. Marie, Ontario, P6A 2E5, Canada, 2Department of Microbiology, University of Guelph, Guelph, ON, N1G 2W1, Canada

Genomics approaches, such as expressed sequence tags (ESTs) and microarrays, present a unique opportunity to study the interactions of an insect and its parasites. More than 35,000 sequence reads have been generated from five cDNA libraries of the epidermis, midgut, fat body, whole larvae and cell line of the spruce budworm, *Choristoneura fumiferana*. These ESTs have been assembled into approximately 8000 unigenes using Phrap program. Annotation of these sequences has been conducted against the protein database Swiss-Prot/TrEMBL and the GenBank of NCBI. The unique sequences have been classified for their molecular functions, cellular locations, and biological processes using Gene Ontology classification system. A spruce budworm EST database has been established. These annotated EST sequences serve as a resource base for gene mining, comparative analysis, and study of insect-virus interactions. DNA microarrays containing 3000 unigenes have been developed and used for analysis of global gene expression during development of the spruce budworm and the gene expression profiles in response to different stresses, such as virus infection. This project is supported by Genome Canada through the Ontario Genomics Institute and Canadian Biotechnology Strategy Fund.

Development and pathway of infection of the entomopathogenic alga *Helicospordium* (Chlorophyta: Trebouxiophyceae)  
Verena-Ulrike Bläské-Lietz and Drion G. Boucias

Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, USA

This study examined the morphogenesis and growth dynamics of different cell phenotypes of *Helicospordium* spp., a unicellular, non-chlorophyll green alga. Regeneration of purified filamentous cells resulted in the release of four oval-shaped daughter cells, which elongated and divided into spherical vegetative cells. These underwent several cycles of autosporation. Media depletion significantly inhibited vegetative growth in vitro. Multiply passaged cultures formed non-motile adherent cells that clustered together via production of extracellular mucilage. Attempts to produce cysts in vitro failed. Vegetative cells reached a maximum density of 1.3 ± 0.3 x 10^5 cells per microliter in 500 μl medium. Cell numbers determined in two experimental noctuid hosts reached densities of 2.2 ± 0.5 x 10^6 and 6.8 ± 2.6 x 10^6 cells per microliter hemolymph in *Spodoptera exigua* and *Helicoverpa zea*, respectively. Cyst morphogenesis in these hosts occurred at high cell densities 4-5 d after hemocoelic injection of 5000 cells per insect. Variable numbers of orally transmitted infectious cysts ruptured in the midgut lumen of the insect, and released the invasive filaments. A small number of filaments passed the midgut epithelium and entered the hemocoel within 4-24 h after cyst ingestion. Within 48 h, vegetative cell stages were detected in the hemolymph.

*STU* Contributed paper. Tuesday, 11:05. 87

*Helicospordium* sp. infection in mosquito larvae  
Tracy M. Conklin1, Verena-Ulrike Bläské, James J. Beene2, and Drion G. Boucias1

1Department of Entomology and Nematology, University of Florida, Gainesville FL 32611, 2United States Department of Agriculture, CMAVE, Gainesville, FL 32604, USA

Members of the genus *Helicospordium* are the first described algal insect pathogens. They have a close affinity to the non-photosynthetic algae of the genus *Prototheca*, and exhibit a wide host range, infecting many species of aquatic and terrestrial insects. In this study, the infectivity of two *Helicospordium* spp. isolates, originating from a black fly (SjHe) and an aquatic weevil (CsHe), was tested against four mosquito species (*Anopheles quadrimaculatus* Say, *Anopheles albimanus* Weidemann, *Culex quinquefasciatus* Say, and *Aedes aegypti* (L.)). Each of these experimental hosts was susceptible to infection with SjHe and CsHe. *Anopheles quadrimaculatus* was the most susceptible mosquito species measured by infection rate and mortality. In *An. quadrimaculatus* exposure to helicosporidia did not effect development of the larvae, whereas in exposed *Ae. aegypti*, larval development was impaired. Bioassays with *Ae. aegypti* also showed that susceptibility to infection decreased with increasing age of larvae. In addition, greater resistance and melanization in all hosts in response to CsHe may indicate that mosquito larvae are less-suitable hosts for this isolate. Future research will be focused on interactions between host and pathogen development and the mechanism of pathogen ingress.

*STU* Contributed paper. Tuesday, 11:20. 88

Identification of genes transcribed by *Moraxella osloensis* in slug *Deroceras reticulatum* using selective capture of transcribed sequences  
Ruisheng An, Sri Sreevatsan, and Parwinder Grewal

Department of Entomology, OARDC, The Ohio State University, OH 44691, USA

Slugs particularly *Deroceras reticulatum* are important pests of agricultural and horticultural plants. The bacterium *Moraxella osloensis* associated with slug-parasitic nematode *Phasmarhabditis hermaphrodita* has potential for the biocontrol of *D. reticulatum*. *P. hermaphrodita* vectors *M. osloensis* into the shell cavity of *D. reticulatum* which multiplies and kills slugs. As *M. osloensis* is the main killing agent, genes expressed by *M. osloensis* in slugs are likely to play important roles in virulence. In this study, selective capture of transcribed sequences (SCOTS) was employed to identify genes expressed by *M. osloensis* in *D. reticulatum* post infection. 11 genes exclusively expressed in the slug by *M. osloensis* were identified. Most of the identified genes are homologous to other bacteria, and function in cell structure, metabolism etc. Other identified genes do not exhibit similarity to any genes or gene products in current databases, and are thus novel. The products of these genes may be crucial determinants of *M. osloensis* virulence. Therefore, the identification of genes expressed by *M. osloensis* in slugs would contribute significantly to the understanding of the virulence mechanism. Characterizing these genes and deciphering their roles should enable us to gain a better understanding of bacterial pathogenesis. Such studies are in progress.

Tuesday, 10:20-12:20

POSTERS – 1

**FUNGI**

Poster / Fungi. F-1

Characteristics and phylogenetic classification of *Cordyceps* and its allies, *Entomopathogenic fungi*  
Sung-Hee Nani1, In-Pyo Hong1, Jae-Sam Hwang2, Seung-Beom Hong1, Sang-Duk Ji1, Seok-Woo Kang1 and Myung-Sae Han2

1Department of Agricultural Biology, National Institute of Agricultural Science Technology, RDA, Suwon 441-100, Korea, 2National Institute of Agricultural Biotechnology, RDA, Suwon 441-100, Korea, 3College of Agriculture and Life Science, Kyungpook National University, Daegu 702-701, Korea

Morphological characteristics of *Cordyceps* species and its allies collected in Korea were clarified and their phylogenetic relationships were also analyzed based on molecular data. *Cordyceps* and its allies of 17 species of 5 genera were identified. Three most common species were *C. nutans*, *P. tenuepse* and *C. militaris*, mainly found early in August when the relative humidity and temperature were high, of which *C. nutans* occupied the highest frequency consisting of 65% in total collections. Neither variation in ascomata arrangement in
stomata nor development of secondary spores was recognizable, while the number, shape and color of stomata varied with insect hosts and weather conditions. ITS 1, ITS 2 and 5.8 S DNA regions amplified from 21 isolates produced a total of 468 to 498 base pairs. The delimitation of genera, Cordyceps, Beauveria, and Paecilomyces were inferred, but intraspecific differentiation was not achieved. Absence of variation in different collections of C. militaris, C. sinensis, B. bassiana, P. tenueipes from different locality implied that environmental factors did not affect the genetic variety. The sequences of C. militaris showed only 81-83% similarity with those of the same generic species, C. sinensis, whereas 91% similarity was shared with those of B. bassiana.

Poster / Fungi. F-2.

RAPD analysis of isolates of Beauveria bassiana, a pathogenic fungus to the silkworm, Bombyx mori L.

Liangen Shi and Jie Jin

College of Animal Sciences, Zhejiang University, Hangzhou 310029, People’s Republic of China

Genetic relationships of 10 isolates of Beauveria bassiana were analyzed with RAPD (Random Amplified Polymorphic DNA)-PCR. The similarity coefficient was initially calculated by using Nei’s formula, and the genetic distance was used to produce UPGMA (Unweighted Pair-Group Method using Arithmetic Average) dendrogram. A total of 138 DNA bands were amplified with 4 random decamer primers, 88 of which were polymorphic bands. The dendrogram showed that the isolates of B. bassiana from Zhejiang University, the Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Anhui Agricultural University, Southwest Agriculture University and Suzhou University were distinctly classified into one category, and the two isolates of B. bassiana from Zhejiang University and Southwest Agriculture University have the nearest genetic relationship. The isolates of B. bassiana from Shandong Agriculture University and the Sericultural Research Institute, Zhejiang Academy of Agricultural Sciences were classified into same category. And the isolate of B. bassiana from Shandong Agriculture University and the Sericultural Research Institute, Zhejiang University, the Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Anhui Agriculture University, Southwest Agriculture University and Suzhou University were distinctly classified into one category, and the two isolates of B. bassiana from Zhejiang University and Southwest Agriculture University have the nearest genetic relationship. The isolates of B. bassiana from Shandong Agriculture University and the Sericultural Research Institute, Zhejiang Academy of Agricultural Sciences were classified into same category. And the isolate of B. bassiana from the Sericultural Research Institute, Guangdong Academy of Agricultural Sciences has great genetic differentiation to other isolates. Furthermore, the dendrogram also showed DNA polymorphism of the isolate that host is Ostrinia furnacalis was most abundant, and the genetic distance was the biggest. The results indicated that genetic variability were presented on different isolates of Beauveria bassiana, and is related to hosts, not to geographic origins.

Characterization of Beauveria bassiana isolates based on ITS and TEF sequences

Reza Talaei-hassanloui1, Aziz Kharazi-pakdel1, Mark S. Goettel2, Javad Mozaffari3 and John Bissett4

1Dept. of Plant Protection, College of Agriculture, University of Tehran, Karaj, 31584-11187, Iran, 2Lethbridge Research Centre, 5401-3 Avenue South, P.O.Box 3000, Lethbridge, Alberta T1J 4B1, Canada, 3Dept. of Genetics, Seed and Plant Improvement Institute, Karaj, Iran, and 4ECORC, Nearby Bldg.21, 960 Carling Ave., Ottawa, Ontario, Canada

Ten Beauveria bassiana isolates from different origins were compared for 5.8 S ribosomal RNA gene and internal transcribed spacer (ITS) and transcription elongation factor (TEF) sequences. DNA sequence alignment of ITS1-5.8 S SITS2 including 595 nucleotids, demonstrated that 0.16-1.77% sequence variation existed among ten isolates. Three distinct groups were determined using UP GMA clustering which slightly correlated with origin of isolates (insect host or soil). Protein coding gene, tef-1a sequence alignment indicated that a 1.8% sequence variation among isolates. Sequencing tef -1a gave three nonambiguous genotypes. There was an obvious correlation between these genotypes and geographic distribution of isolates. No significant correlation was found between B. bassiana genotypes and pathogenicity against diamond back moth, Plutella xylostella and Colorado potato beetle, Leptinotarsa decemlineata.

Characterization of entomopathogenic fungus Paecilomyces fumosoroseus to common white Pieris rapae crucivora

Hajime Hiromori, Dai Yaginuma, Natsumi Washizu and Mami Kimura

Department of Applied Entomology, Faculty of Agriculture, Shizuoka University, Ohya 836 Shizuoka, 422-8529, Japan

Common white larvae is one of the important insect pests of many species of Brassica. However, many other species of pests damage to Brassica. To develop a new microbial insecticide, various entomopathogenic fungi were assayed to common white. Paecilomyces fumosoroseus (strain: SPF-1) isolated from soil showed high pathogenicity to common white larva. Conidal suspension (1.0 x 106 conidia/ml) of SPF-1 resulted 90% mortality. In general, entomopathogens have specificity to host insects. This specificity restricted the utilization of entomopathogen as useful control agent. We reported the SPF-1 had high pathogenicity to green peach aphid Myzus persicae. In this research, we treated SPF-1 to diamond-back moth Plutella xylostella. SPF-1 showed high pathogenicity to P. xylostella. From these results, SPF-1 has possibility the useful control agent to pests of Brassica at the same time.

Characterization of entomopathogenic fungi of oca weevil Adioristidius tuberculatus Voss in the Andean region of Peru

J. Salazar1, V. Cátedra1, J. Alcázar1 and A. Lagnaoui2

1International Potato Center (CIP), Lima, Peru, 2The World Bank, Environmentally and Socially Sustainable Development, Washington DC, USA

The oca weevil, Adioristidius tuberculatus Voss is the most important pest of the Andean oca tuber crops (Oxalis tuberosa Molina). Entomopathogenic fungi offer good possibilities as control agents in Integrated Pest Management (IPM) strategies. Fungi were isolated from weevils collected from fields and storages in Junín, Peru. These were identified and characterized morphologically and physiologically in the laboratory. Natural infection rates were 10% and 12% for the oca weevils in the both field and storage. Nine fungi
strains were isolated and identified as *Paecilomyces fumosoroseus*, *Beauveria bassiana* and *Beauveria brongniartii*. Pathogenicity tests revealed high rates of infection (>90% in larvae-IV and >80% in adults) for the isolates CIP76 *P. fumosoroseus*, CIP78 *B. brongniartii*, CIP81 *B. bassiana* and CIP83 *P. fumosoroseus* in oca weevils. The isolate CIP76 *P. fumosoroseus* highest efficacy against the oca weevil (Larvae: LC50 = 1x10⁶ conidias ml⁻¹, TL50 = 7 days; adults: LC50 = 1,3x10⁷ conidias ml⁻¹ and TL50 = 29 days). The morphological characterization of the three species showed that the size of their conidia were outside of the range that is characteristic for each species. CIP76 *P. fumosoroseus* was the most virulent isolates with good multiplication of conidia, which make them very promising for usage in Integrate Pest Management strategies of *A. tuberculatus*.

**STU** Poster / Fungi. F-7.

Factors relating to epizootics of *Hirustella sp.* in field populations of *Homalodisca coagulata* (Say) (Homoptera: Cicadellidae)

S. E. Breaux¹, D. G. Boucias¹, R. F. Mizell III²

¹Univ. of Florida, Department of Entomology and Nematology, Gainesville, FL 32611-0680, USA, ²Univ. of Florida, North Florida Research and Education Center, Quincy, FL 32351-5677, USA

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Homoptera: Cicadellidae), is a highly polyphagous xylephage known to feed on over 100 different species of plants. Native to the Southeastern U.S., it is most notable for its introduction and subsequent spread in California and its efficiency as a vector of *Xylella fastidiosa*, a phytopathogenic bacterium that is the causative agent of a number of important plant diseases. Research was undertaken in *H. coagulata*’s native range to identify naturally-occurring entomopathogens. Three possible organisms were found, the most prevalent of which was a novel species of *Hirustella*, which has been designated *H. homalodisca*. A crape myrtle field plot was utilized to track a coexisting population of both the host and pathogen over the course of the summer epizootic, as well as the effect of humidity on this population. Hemolymph bleeds were utilized to provide population percent infection data and to investigate age-based variations in the susceptibility of host to pathogen. Investigation into the host/pathogen dynamics of the *H. coagulata/H. homalodisca* system and the effect of humidity on this relationship is the first step in the evaluation of the worth of the *Hirustella* mycopathogen as a potential biocontrol agent for use against GWSS in non-native ranges.

Poster / Fungi. F-8.

Growth and virulent characteristics of *Verticillium lecanii* (*Lecanicillium spp.*) hybrid strains

D. Arioli¹, M. Koike¹, K. Inami¹, Y. Baba¹, M. Sugimoto²

¹Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Hokkaido, Japan, ²Okinawa Prefectural Agricultural Experiment Station, Naha 903-0814, Japan

Mass-produced biocontrol agents Mycotal and Vertalec have a high effective methods to breeding the entomopathogenic fungi. We also report screening of virulence to aphid and whitefly.

**STU** Poster / Fungi. F-9.

The generalist predator *Anthocoris nemorum* detects and avoids *Beauveria bassiana*

Nicola V. Meyling¹ and Judith K. Pell²

¹Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldensvej 40, DK-1871 Frederiksberg C, Denmark, ²Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2QJ, UK

Limited knowledge is available of whether insects can assess cues related to entomopathogenic fungi which can cause significant mortality in their populations. In laboratory bioassays we investigated the ability of the generalist predator *Anthocoris nemorum* L. (Heteroptera: Anthocoridae) to detect the presence of its natural enemy, the fungal pathogen *Beauveria bassiana*. Behavioural observations were conducted on adults of *A. nemorum* foraging in choice and non-choice arenas treated with conidial suspensions of *B. bassiana* or just the carrier. The arenas consisted either of nettle leaves or soil. Additionally, behaviours in response to sporulating nettle aphid cadavers compared to uninfected aphids or paper balls were evaluated on nettle leaves. An oviposition experiment was also conducted in choice arenas on nettle leaves. Predators detected and avoided contact with leaf surfaces inoculated with *B. bassiana*. Females that were forced to enter fungus treated leaf surfaces were very reluctant to do so. When females encountered cadavers sporulating with *B. bassiana* they rapidly withdrew compared to harmless paper ball dummies. Soil inoculated with *B. bassiana* did not affect *A. nemorum* behaviour or residence time compared to control soil. Females inserted significantly more eggs in control leaf areas than areas treated with *B. bassiana* conidia.

Poster / Fungi. F-10.

Interactions between over-wintering seven spot ladybirds (*Coccinella septempunctata*) and the entomopathogenic fungus *Beauveria bassiana*: The 12 buckets

Emma Ormond¹, Alison Thomas¹, Judith Pell² and Helen Roy¹

¹Department of Life Sciences, Anglia Polytechnic University, Cambridge, CB1 1PT, UK, ²Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, AL5 2QJ, UK

Adult seven spot-ladybirds (*Coccinella septempunctata*) spend the winter months in a dormant state, often in the leaf litter. It is widely reported that *Beauveria bassiana* is a significant factor in their winter mortality, however this has never been quantified. In this presentation I will describe semi-field experiments designed to ascertain the prevalence of *B. bassiana* in overwintering seven-spot ladybird populations. In mid October (early winter) field collected seven spot ladybirds were placed in 12 buckets, which were subsequently placed outside under semi-field conditions to determine winter mortality due to *B. bassiana*. On three occasions, early winter (mid November), late winter (mid January) and early spring (mid March) sub-samples of the ladybird population were destructively sampled and incubated in the laboratory at 22°C and mortality was recorded over a two-week period. Soil samples were also assessed for the presence of *B. bassiana* using a combination of serial dilution plating on to selective media and *Galleria mellonella* baiting. Isolates of *B. bassiana* obtained by these processes were investigated using Inter Simple-Sequence Repeat (ISSR) PCR to establish both the number of isolates present and also the percentage which were infective to seven spot ladybirds. Initial results indicate that 4-6% of ladybird mortality is due to *B. bassiana*. Interestingly whilst most ladybirds formed aggregations those which were dead on collection were almost invariably lone regardless of whether or not sporulation was occurring. These results will be discussed both from an ecological perspective and with particular consideration of the diversity of *B. bassiana* isolates indicated by the molecular studies.
Comparison of Galleria baiting and soil plating methods for isolating soilborne pathogens from the habitats of glassy-winged sharpshooter, Homalodisca coagulata (Heteroptera: Cicadellidae) in California

Surenda K. Dara¹, Michael R. McGuire² and Harry K. Kaya¹

¹Shafter Research and Extension Center, Shafter, CA 93311 ²USDA-ARS, Shafter, CA 93311, ¹Department of Nematology, University of California, Davis, CA 95616, USA

In search of suitable entomopathogenic fungi for the control of the glassy-winged sharpshooter (GWSS), Homalodisca coagulata, soil samples were collected from citrus and pomegranate orchards in Tulare and Riverside counties in California. Using a modified Galleria bait method that required a small quantity of soil, 78 isolates of Beauveria bassiana were isolated compared to soil plating on a selective medium that gave rise to 22 isolates. In a different assay, using Galleria bait method, 37 isolates of B. bassiana were obtained from an organic citrus orchard while 3 isolates of B. bassiana and 4 isolates of Metarhizium anisopliae were obtained from a conventional pomegranate orchard.

Comparative susceptibility of Metarhizium anisopliae varieties anisopliae and acridum to the selective fungicide dodine

Seth J. Dettenmaier, Drauzio E. N. Rangel, Edward W. Evans, Donald W. Roberts

Department of Biology, Utah State University, Logan, UT 84322-5305, USA

The fungicide dodine is widely used in selective media for the isolation of entomopathogenic fungi. In this study, five isolates of two varieties of Metarhizium anisopliae were tested (M. a. var. anisopliae ARSEF 2575, ARSEF 3609, ARSEF 5749 and M. a. var. acridum var. acridum ARSEF 324, ARSEF 3341) for sensitivity to concentrations of dodine ranging from 0.0001 to 0.01% active ingredient (A.I.). Two sources of dodine, either Syllit® commercial fungicide (65% A.I.) or Sigma® reagent grade (100% A.I.), were added to Czapek agar medium (an inorganic-nitrogen-based medium). A conidial suspension (ca. 10⁶ conidia/ml) were dropped on the medium surface, and conidial germination was recorded at 24, 48, and 72 hours at 400x magnification. Responses to Syllit® and Sigma® preparations were virtually identical for each isolate. At 24 hours, the M. a. var. acridum isolates were inhibited by concentrations of 0.0003%; and at 72 hours, germination was almost completely repressed at concentrations of 0.004% and above. M. a. var. anisopliae isolates, except for 3609, were much less sensitive to dodine. ARSEF 3609 was the most sensitive of all isolates tested, e.g., even after 72 hours germination was almost completely inhibited at 0.001%. All isolates were originally isolated from grasshoppers with the exception of ARSEF 2575 which came from a coleopterous insect. Although ARSEF 3609 is classified as an M. a. var. anisopliae by molecular methods and is closer to M. a. var. acridum in colony growth and color, UV-B and heat tolerance, as well as dodine susceptibility. The high vulnerability of some of the tested isolates to low concentrations of dodine in Czapek medium indicates that this compound will not be effective in selective media designed for their isolation from soil and other contaminated substrates.

Virulence of two Metarhizium anisopliae varieties to Mormon cricket, Anabrus simplex, nymphs and adults

Helen G. Bignay¹,², Drauzio E. N. Rangel¹, Edward W. Evans¹, and Donald W. Roberts³

¹Department of Biology, Utah State University, Logan, UT 84322-5305, USA, ²Bureau of Plant Industry, National Mango Research and Development Center, Jordan, Guimaras 5045, Philippines

The Mormon cricket, Anabrus simplex (Orthoptera: Tettigoniidae), has a long and negative history in Utah and other USA western states. This insect, although flightless, often migrates in bands of thousands of individuals; and their feeding can cause significant damage to rangeland and forage plants as well as cultivated crops. In the present study, we tested one isolate of Metarhizium anisopliae var. anisopliae (ARSEF 2575) and one isolate of M. anisopliae var. acridum (ARSEF 324) for virulence to nymphs of 6th and 7th instar and adult Mormon crickets. The insects were immersed in grasshopper meal for a period of six in a suspension of 1 x 10⁹ conidia/ml 0.1% Tween 80 for 15 seconds. The controls (untreated) were dipped in Tween 80 (0.1%). Immediately after treatment, the insects were transferred individually into glass jars with a fine layer of sterile white sand and provided with food (50:50 wheat bran and Flicker’s high calcium cricket feed) and saguaro leaves. The jars were held in high-humidity chambers (plastic boxes lined with water-soaked filter paper) in a 28°C incubator. Mortality was assessed daily; and dead insects collected, surface sterilized and incubated in humid chambers. Development of the fungus on the cadaver was assessed as confirmed mycosis. Thirty insects were used for each fungal treatment. Three trials were conducted with 6th and 7th nymphs; and two trials were conducted with adults. ARSEF 2575, with a LT₅₀ of 3.42 and 3.25 days for nymphs and adults, respectively, was somewhat more virulent to Mormon cricket than isolate ARSEF 324 with a LT₅₀ of 4.42 and 5.13 days for nymphs and adults, respectively. Both isolates afforded 100% mortality and approximately 60% confirmed mycosis at 7 days. The results indicate that these isolates are virulent for both nymphal and adult stages of the Mormon cricket; and, with further development, may have potential for use in integrated pest management systems (IPM) for control this pest.

Isolates of Metarhizium anisopliae are diverse in their relationships between pigments and stress tolerance

Drauzio E. N. Rangel¹, Gilberto U. L. Brajao¹, Anne J. Anderson¹ and Donald W. Roberts³

¹Department of Biology, Utah State University, Logan, UT 84322-5305, USA, ³Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, 14040-903, Brazil

Conidial pigmentation is involved in protection against heat and ultraviolet radiation in several fungi. In this study, we compared the tolerance to wet-heat (45°C, for 2, 4, and 6 h) and two kinds of ultraviolet radiation (at total dose of 7.14 kJ/m²) of 17 color mutants of wild-type ARSEF 23 and 13 color mutants of wild-type ARSEF 2575 of Metarhizium anisopliae var. anisopliae. The stress tolerance of all mutants were compared with that of their wild-types, and with the most thermo- and ultraviolet-tolerant wild-type we have tested to date, viz. ARSEF 324, an M. a. var. acridum. The color of each isolate or mutant was identified using the PANTONE Color Standard (Eiseman and Herbert, 1990). In addition, the pigments of each mutant or wild-type were extracted and their UV absorbances compared with each strain’s tolerance to both stresses. No correlations were detected. Color mutants of ARSEF 23, in general, were less UV tolerant than their parent wild-type. The conidial pigmentation is important for conidial tolerance to UV radiation for ARSEF 23, but less so for ARSEF 2575. The ARSEF 2575 color mutants demonstrated less variability in UV tolerance than those of ARSEF 23, even though very similar colors were presented in the two groups of mutants. Color mutants when reverted to wild-type color conidia recovered wild-type UV tolerance. For thermotolerance, however, mutants in several instances were more tolerant than their wild-types parents. Accordingly, the darker pigmentation of wild-types did not provide protection against heat.

Are ‘stressed-out’ wireworms more susceptible to the biocontrol agent Metarhizium anisopliae?

Jerry Eriksen¹,², J. Todd Kabaluk³, Mark Goettel³, Murray Isman³, Eduardo Jovel¹, Judith H. Myers³

¹Faculty of Agricultural Sciences, 2357 Main Mall, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, and Agriculture and Agri-Food Canada, ²Pacific Agricultural Research Centre, Box 1000, Agassiz, British Columbia, V0M 1A0, Lethbridge Research Centre, Box 3000, Lethbridge, Alberta, T1J 4B1, Canada
The entomopathogenic fungus *Metarhizium anisopliae* (M.a), is a candidate for use in the biological control of two noxious wireworm species, *Agriotes obscurus* (A. o), and *A. lineatus* (A. l) yet bioassays with both species have revealed vast differences in *M.a* susceptibility. Features of the wireworm immune system were investigated to identify important factors that contribute to differences in susceptibility found within a population of wireworms. After *M. a* exposure, (1) hemolymph protein composition was analyzed via MALDI-TOF mass spectrometry, (2) prophenoloxidase activity was determined, and (3) hemocyte densities were enumerated and compared. Trace amounts of inducible peptides were detected via MALDI-TOF MS, whereas reductions in prophenoloxidase activity occurred after *M.a* exposure. Total hemocyte counts were not different after exposure; but the hemocyte-composition was significantly different from that of control wireworm. Insecticides are known to compliment the effect of *M.a* in other insect orders, therefore ‘stress’ bioassays that utilize three biorational pesticides in combination with *M.a* were evaluated for mortality. Spinosyns were synergistic with *M.a*, at 10X the rate, whereas halofenozide and the clove-oil / thyme-oil treatments displayed additive mortality effects with *M.a*. These preliminary results suggest that stress from insecticidal activity can increase the wireworm’s susceptibility to *M.a*.

**Influence of plant rhizosphere on the abundance of entomopathogenic fungi**

Daniel R. Sosa-Gómez, Alvaro M. R. Almeida, Jose J. da Silva and Luiz C. Benato

Embrapa Soja. Cx.P. 231 Londrina, PR, 86001-970, Brazil

*Beauveria bassiana*, *Paecilomyces* sp. and *Metarhizium anisopliae* are cosmopolitan fungi that are found in agricultural and forest soils. We report the influence of the rhizosphere of soybean, wheat, sunflower, corn, wild radish (*Raphanus sativus*) and lupine (*Lupinus albus*) on the abundance of colony forming units (CFU) of these entomopathogenic fungi. Soil samples were collected from the rhizospheres of each plant species and from soil outside of the root projection zone. Soil samples were suspended in water and a serial dilution was plated on dodeine-based medium. CFUs were quantified after 15 days of incubation. The density of *B. bassiana* ranged from 0 to 1,442 CFU/g dry soil with greater densities in the rhizosphere than in the outer rhizosphere of all plant species, in most of the cases. The numbers of *M. anisopliae* CFUs did not follow a consistent pattern like *B. bassiana*. The numbers of *Paecilomyces* sp. CFUs from the rhizosphere did not differ from CFU numbers outside of the root projection zone. No differences of CFUs were found by plant species. These results suggest that chemical (root exudates) and physical (humidity, aeration) conditions near the roots can influence the prevalence of propagules of certain species of entomopathogenic fungi.

**Colonization of sugarbeet roots by entomopathogenic fungi**

Cindy Fuller-Schaefer1, Kerstin Jung2, and Stefan Jaronski1

1United States Department of Agriculture, Agricultural Research Service, Northern Plains Agricultural Research Laboratory, Sidney, MT 59270, USA.
2Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany

The fungus *Metarhizium anisopliae* is currently being developed as a biocontrol agent of *Tetanops myopaeformis*, the sugarbeet root maggot. This insect damages roots directly by surface feeding on roots. The feeding scars can be further exploited by pathogenic/opportunistic microorganisms which cause additional damage to the sugarbeet as well as potentially play a symbiotic role with the insect. Application of fungi as a seed coat for subsequent colonization of the root could provide an ideal system for control because the fungus would be specifically present in the maggot habitat. The ability of *M. anisopliae*, as well as *Beauveria bassiana*, to colonize sugar beet roots was investigated in gnotobiotic systems as a preliminary to studying biotic and abiotic factors affecting colonization. Using polyethylene glycol-based transformation system, two isolates of *M. anisopliae* (MA1200 and F52) and one *B. bassiana* (GHA) were co-transformed with pTEFE GFP, which encodes the red-shifted green fluorescent protein variant EGFP, and pBENA3, which confers resistance to the fungicide benomyl. After stable transformants were selected, expression of EGFP was confirmed by fluorescence microscopy and Western blotting with mouse anti-GFP. Effects of transformation on growth and virulence were also examined. Colonization of roots by EGFP-expressing fungi was observed using traditional fluorescence microscopy and laser scanning confocal microscopy.

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**Challenges and constraints in deploying *Metarhizium anisopliae* for biocontrol of sugarbeet root maggot, *Tetanops myopaeformis***

Stefan T. Jaronski, Julie A. Grace, and Rob Schlottauer

USDA ARS Northern Plains Agricultural Research Laboratory, Sidney, MT, USA

The entomopathogenic fungus, *Metarhizium anisopliae*, Strain MA1200 has been under study by USDA ARS and North Dakota State University as a potential mycoinsecticide for controlling the root maggot, *Tetanops myopaeformis*. These fungi can be used in the face of high insect pressure but needs integration with other tools. The fungus is not efficacious by itself in proportional to soil moisture, but soil type and moisture interact to complicate this relationship. The fungus is not efficacious by itself in proportion to soil moisture, but soil type and moisture interact to complicate this relationship. The fungus is not efficacious by itself in proportion to soil moisture, but soil type and moisture interact to complicate this relationship. The fungus is not efficacious by itself in proportion to soil moisture, but soil type and moisture interact to complicate this relationship.

**Observations on the interaction between biocontrol fungi, *Metarhizium* and *Beauveria*, and bacteria isolated from the rhizosphere of sugar beets***

Kerstin Jung1, Cindy Fuller-Schaefer2, Ben Larson1 and Stefan T. Jaronski2

1Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany, 2USDA-ARS Northern Plains Agricultural Research Laboratory, Sidney, MT, USA

USDA-ARS is developing a biocontrol strategy for the sugar beet root maggot, *Tetanops myopaeformis* using entomopathogenic fungi (EPF). One approach is to have EPF colonize the surface of the developing sugar beet tap roots in advance of the insect, but soil micro-organisms may interfere with this process. We therefore examined the in vitro effect of rhizosphere/rhizoplane bacteria on several strains each of *Beauveria bassiana* and *Metarhizium anisopliae*. Micro-organisms were isolated from surfaces of field collected sugar beets using different media. Of a total of 200 bacteria, 40 (based on colony morphology and Gram-reaction) plus 4 bacteria being developed to control sugar beet pathogens, were chosen to study their interactions with different isolates of EPF. The interaction was studied by cross-streaking the bacteria through a line of fungal mycelium on two different media. Differences between and within the two genera of EPF were observed, e.g. with some bacteria growth was inhibited by most *Metarhizium* but not *Beauveria*-isolates. None of the bacteria studied inhibited all of the fungi tested. On Potato Dextrose Agar the fungi had a greater inhibitory effect on the bacteria compared to Plate Count Agar. The significance of these observations for biocontrol of soil-dwelling insects will be discussed.

**Poster / Fungi. F-16.**

**Poster / Fungi. F-17.**

**Poster / Fungi. F-18.**

**Poster / Fungi. F-19.**
Coffee endophytes pathogenic to the coffee berry borer

Francisco Posada and Fernando E. Vega

Insect Biocontrol Laboratory, U. S. Department of Agriculture, Agricultural Research Service, Bldg. 011A, BARC-W, Beltsville, Maryland 20705, USA

The coffee berry borer, Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae) is responsible for enormous economic losses to coffee growers throughout the world. Due to its life cycle, which is spent mostly inside the coffee berry, the insect is very difficult to manage. In an attempt to develop novel methods against this insect, we have been able to inoculate coffee seedlings with Beauveria bassiana. In the process of assessing what endophytes are present in mature coffee plants - which might interfere with B. bassiana establishment - we sampled coffee tissues in Colombia, Hawaii, Mexico and Puerto Rico, and among more than 1200 isolates we have identified four endophytic fungi that have been proven to be pathogenic to the coffee berry borer: (1) Beauveria bassiana isolated from the peduncle of coffee berries in Colombia; (2) Clonostachys rosea isolated from coffee leaves in Colombia; (3) Acremonium alternatum isolated from the pulp of coffee berries in Colombia and (4) Fusarium sp. isolated from coffee flowers in Hawaii. In addition, we have isolated a new species of Metarhizium as a coffee endophyte in Hawaii, Colombia, and in coffee seedlings purchased at a nursery in Maryland. Members of this genus have been reported to be pathogenic to mites. Our results point out the wealth of endophytic diversity in coffee tissues and at the still unsolved mystery of what function these fungi might have. Could fungi previously unreported as entomopathogens but present as endophytes serve as new fungal biocontrol agents?

Poster / Fungi. F-20.

Coffee endophytes pathogenic to the coffee berry borer

Francisco Posada and Fernando E. Vega

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The coffee berry borer, Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae) is responsible for enormous economic losses to coffee growers throughout the world. Due to its life cycle, which is spent mostly inside the coffee berry, the insect is very difficult to manage. In an attempt to develop novel methods against this insect, we have been able to inoculate coffee seedlings with Beauveria bassiana. In the process of assessing what endophytes are present in mature coffee plants - which might interfere with B. bassiana establishment - we sampled coffee tissues in Colombia, Hawaii, Mexico and Puerto Rico, and among more than 1200 isolates we have identified four endophytic fungi that have been proven to be pathogenic to the coffee berry borer: (1) Beauveria bassiana isolated from the peduncle of coffee berries in Colombia; (2) Clonostachys rosea isolated from coffee leaves in Colombia; (3) Acremonium alternatum isolated from the pulp of coffee berries in Colombia and (4) Fusarium sp. isolated from coffee flowers in Hawaii. In addition, we have isolated a new species of Metarhizium as a coffee endophyte in Hawaii, Colombia, and in coffee seedlings purchased at a nursery in Maryland. Members of this genus have been reported to be pathogenic to mites. Our results point out the wealth of endophytic diversity in coffee tissues and at the still unsolved mystery of what function these fungi might have. Could fungi previously unreported as entomopathogens but present as endophytes serve as new fungal biocontrol agents?

Poster / Fungi. F-20.

Low likelihood of recombination between the introduced Beauveria bassiana strain GHA and indigenous conspecific strains based on vegetative compatibility groupings

Louella A. Castrillo1, Seanna L. Annis2, Eleanor Groden1, Prashant K. Mishra2, and John D. Vandenberg3

1Dept. of Entomology, Cornell University, Ithaca, NY, 2Dept. of Biological Sciences, University of Maine, Orono, ME 04469, 3USDA-ARS, US Plant, Soil and Nutrition Lab., Tower Road, Ithaca, NY 14853, USA

Among fungi with no known sexual stage or are predominately haploid, the parasexual cycle provides a means of DNA exchange and recombination as has been shown in a wide range of asexual fungi. During the parasexual cycle vegetatively compatible hyphal fusi allowing exchange of genetic material and recombination. Genetic recombination is an important component of risk assessment studies of entomopathogens that are sprayed repeatedly in large quantities in agricultural fields as microbial control agents. The likelihood of recombination and the impact of resulting recombinants on non-target organisms need to be considered because strain virulence and host range are critical to assessing safety. We assessed the likelihood of recombination between the commercial strain Beauveria bassiana GHA and indigenous conspecific strains by determining vegetative compatibility groups present in fields in Maine and New York with various histories of GHA application. Thirty-seven strains out of 110 soil isolates characterized using AFLP and RAPD markers were selected to represent the different cluster groups of indigenous and GHA-similar genotypes observed. Results showed that strains from all three genetic clusters of indigenous populations found in Maine and New York were vegetatively incompatible with GHA, indicating low likelihood of parasexual recombination between the introduced and indigenous strains.

Poster / Fungi. F-21.

Purification and gene cloning of a new hydrophobin-like protein that relates to thermal tolerance of aerial conidia of fungal biocontrol agents

Sheng-Hua Ying1 and Ming-Guang Feng1,2

1Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China, 2Institute of Applied Entomology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China

The contents of hydrophobin-like or formic-acid-extractable (FAE) proteins in aerial conidia produced on solid substrate varied greatly among the 14 isolates of Beauveria bassiana (Bb), Paecilomyces fumosoroseus (Pf) and Metarhizium anisopliae (Ma) as fungal biocontrol agents, ranging from 5.0 µg/mg of Ma 297 to 23.4 µg/mg of Bb 2861. Based on SDS-PAGE analysis, the FAE components differed among the fungal species, including mainly 12.0, 15.0 and 17.5 kDa in six Bb isolates, and 15.0 and 17.5 kDa in five Pf isolates. It was shown that 80% of variability in conidial thermosterlance was attributed to either 15.0- or 17.5-kDa FAE protein or both. However, the FAE components diversified in three Ma isolates, i.e., 17.0 and 14.5 kDa in Ma 2125, 19.0 and 13.5 kDa in Ma 0201, and only 13.5 kDa in Ma 297. Moreover, the FAE proteins from aerial conidia, aerial mycelia and submerged mycelia of Bb 2860 grown on Sabouraud dextrose agar or broth differed significantly in quantity (26.3, 18.2 and 16.5 µg/mg) from one to another but primarily 15.0- and 17.5-kDa molecules. The 15.0-kDa protein was purified and its gene was cloned. This protein was confirmed as new in peptide-mass fingerprint and sequence analysis and might play a role in fungal tolerance to thermal stress.

Poster / Fungi. F-23.

Toxins are overproduced in a gene disruption mutant of Metarhizium anisopliae

Stuart B. Krasnoff1, Yong-Sun Moon1, Bruno G.G. Donzelli2,3, Alice C.L. Churchill1,2, John D. Vandenberg1, Donna M. Gibson1

1Department of Plant Pathology, Cornell University, Ithaca, NY, 2Boyce Thompson Institute, Ithaca, NY, 3USDA-ARS, Plant Protection Research Unit, Ithaca, NY, USA

A Metarhizium anisopliae mutant (B1-3) in which a nonribosomal peptide synthetase (NRPS) gene was disrupted produces destruxins and exhibits a marked increase in the production of a complex mixture of secondary metabolites not previously described from this fungus. Overproduction of these compounds in B1-3 imparts a yellow pigmentation to the culture medium of the fungus. This change in phenotype is correlated with a second, uncharacterized mutation distinct from the NRPS gene disruption. Using ESI-MS, as well as 1- and 2-D NMR spectral analyses, we identified two major components in the pigment mixture, NG-391 and NG-393, which were previously reported from Fusarium species as stimulants of nerve-cell growth. These compounds are closely related to another family of Fusarium metabolites, the fusarins, mutagenic mycotoxins that contaminate corn. They consist of a 3-alkenoic acid, 4-poxy-2-pyridinone moiety attached to a substituted pentaene unit that isomerizes readily, especially upon exposure to light.


A study of the expression profile of pathogenicity related genes in the entomopathogenic fungus Beauveria bassiana on different insect cuticles

P. Akbar Ali Khan1, K. Uma Devi1 and Annette Reincke2

1Department of Botany, Andhra University, Visakhapatnam, 530 003 AP India, 2Department of Entomology, Max-Planck Institute of Chemical Ecology, Jena, Germany

Beauveria bassiana Bals. Vuillemin is the most popular among the registered mycoinsecticides. One of the principal reasons for its popularity is its very wide host range of ~750 insect species. Thus it can be used for management of the myriad insect pests of a crop plant. Identification of genes expressed during pathogen-host interactions (cuticular penetration) are of interest as they help to understand the genetic basis of pathogenicity and spot genes if any expressed during infection of particular insects. A study in this direction is being carried out taking the cuticle from the pathogenicity related genes identified in another entomopathogenic fungus Metarhizium anisopliae by St Leger et al (2003)(NCBI). Specific primers for 20 of the M. anisopliae pathogenicity genes and degenerate primers for four genes were designed. The presence of these genes in B. bassiana is confirmed through amplification from
the DNA samples and sequencing of the amplified products. To facilitate the study of genes expressed during host-pathogen interactions, induction of appressoria (infection peg) in *vitro* is achieved through culture of the fungus on cuticle from different insects - *Helicoverpa armigera*, *Spodoptera litura*, *Chilo partellus*, *Mylabris pustulata* and *Periplaneta americana*. The expression profile of the selected genes is being analysed through PCR amplification from the RNA samples obtained from cultures grown on different insect cuticles (as the sole nutrient source).

Poster / Fungi. F-25.

**Some Beauveria bassiana proteinases as one of the determinants of entomopathogenicity**

U. Iskandarov, Anaida Guzalova
Institute of Microbiology, Academy of Sciences of Uzbekistan, 700128, Tashkent, Uzbekistan

Fungi were the first entomopathogenic microorganisms to be recognized as sources of the insect diseases. At present, insect killing fungi have attracted the attention of scientists as potential agents for biological pest control. It is well known the degradation of insect cuticle caused by fungi is the most important step for beginning of the infection process. Using the polycaryaamide gel electrophoresis we had identified *Beauveria bassiana* strains ALG produces PR1 and PLB2 proteinase. These proteinases are synthesized when fungus is cultivated on the Colorado potato beetle cuticle or different types of poor media with cuticle as the addition. In the process of work two kinds of media were prepared. The first medium included the cuticle (0.8% w/v) with basal salt medium based on carbon and nitrogen sources. The second medium did not include the cuticle. Proteinase PR1 was not detected both extra-cellular and intra-cellular when the fungus was grown in the second medium. Proteinase PLB2 was detected in both experimental medium. Both proteinases could be detected as intra- and extra-cellular substances in *B. bassiana* cultures which were grown helping the medium with insect cuticle. The proteinases can be discovered starting from four to six hours after media inoculation. Based on these results we can make conclusion, that the fungus has used extra-cellular protease for utilization of the host. It is the beginning of productive relationship in host-pathogen system. Subsequent development of infection process is connected with establishment of nutritional relationship of fungus with the host. The synthesis of proteolytic enzymes in media containing host cuticle can be used as indicator of infection activity of fungi.

**Targeted disruption of a peptide synthetase gene in* Metarhizium anisopliae* has no effect on destruxins production or virulence against insects**

Yong-Sun Moon1, Stuart B. Krasnoff2, Bruno G.G. Donzelli1,2, John D. Vandenberg1, Donna M. Gibson1, and Alice C.L. Churchill1,2

1Boyce Thompson Institute, Ithaca, NY, 14853, 2Department of Plant Pathology, Cornell University, Ithaca, NY, 14853, 3USDA-ARS, Plant Protection Research Unit, Ithaca, NY, 14853, USA

The principal toxins produced in fermentation by *M. anisopliae* are the destruxins, cyclic depsipeptides with chemical features suggesting synthesis by a nonribosomal peptide synthetase (NRPS). We targeted for further study an NRPS gene fragment (ma267) identified by Freimoser et al. (2003) as an EST expressed after 24 hr of fungus growth on insect cuticle-containing medium. Ma267 detects DNA polymorphisms that correlate with relative levels of *in vitro* destruxins production in 16 *M. anisopliae* isolates. Additionally, ma267 gene expression is positively correlated with *in vitro* destruxins production. We disrupted the ma267 gene by *Agrobacterium tumefaciens*-mediated transformation and identified several stable knockout (KO) transformants. Three KO transformants exhibited normal growth rates and levels of destruxins production comparable to an ectopic transformant and the wild type strain, suggesting that the ma267 gene is not involved in destruxins production. A fourth KO transformant (B1-3) has an additional uncharacterized mutation correlated with overproduction of metabolites not previously reported from *Metarhizium*. We observed no detectable differences in pathogenicity of the four ma267 KO mutants in bioassays against beet armyworm and Colorado potato beetle. Further studies are required to determine whether the ma267 NRPS plays a role in the biology of *M. anisopliae* as an insect pathogen.

**BACTERIA**

**Poster / Bacteria. B-1. Vip3Ba1: a novel Vip protein from *Bacillus thuringiensis***

Cecile Rang1, Patricia Gil2, Nathalie Neisner1, Jeroen Van Rie1 and Roger Frutos2

1Bayer BioScience N.V. Technologiepark 38, 9052, Gent, Belgium, 2CIRAD, UMR 17, IRD-CIRAD, CIRAD TA 207/G, Campus International de Baillarguet, 34398 Montpellier cedex 5, France

A novel vip3-related gene was identified in *Bacillus thuringiensis*. This novel gene is 2406 bp long and codes for a 91-kDa protein (801 aa). This novel protein shares between 60 and 61% similarity with Vip3A proteins and is designated Vip3Ba1. Vip3Ba1 displays several specific features. Differences between Vip3Ba1 and Vip3A proteins are spread throughout the sequence but are more frequent in the C-terminal part from amino acid 456 onwards. The signal sequence is more closely related to that of Vip3A1 than that of Vip3A. The regions containing the two processing sites, highly conserved among the Vip3A toxins, are markedly different in Vip3Ba1. The pattern DCCEE (Asp Cys Cys Glu Glu) is repeated four times between position 463 and 483 in Vip3Ba1 to generate the sequence 463-DCCEEEDCCEEEDCCEEEDCCEE-483. This sequence, rich in negatively charged amino acids, also contains 73% of the cysteines present in Vip3Ba1. This repeated sequence is not present in Vip3A proteins. The protein was produced in *E. coli* and tested against *Ostrinia nubilalis* and *Plutella xylostella* and generated significant growth delay but no larvicidal effect indicating that its host range might be different than that of Vip3A proteins.

**Identification of vip genes in *Bacillus thuringiensis* strains by PCR-RFLP**

Carmen Sara Hernández1, Annemie Boets2, Jeroen Van Rie2, and Juan Ferré1
1Departament de Genètica, Universitat de València, 46100 Burjassot, Spain, 2Bayer BioScience N.V., 9052 Gent, Belgium

By means of a PCR-RFLP strategy, screening of a collection of 507 strains of *Bacillus thuringiensis* has been performed in order to identify known vip genes and to detect potentially novel vip genes. The observed frequency of genes belonging to vip1 and vip2 families was around a 10%, whereas 48.9% of the strains showed amplification of vip3 genes. Following a first positive amplification, 18 strains did not show any amplification product after a second PCR with “typing” primers, suggesting that these strains could contain novel vip genes. Upon digesting the amplicons, four restriction patterns were found within the vip1 family: vip1Aa1, vip1Ba1/vip1Ba2, vip1Ca1 patterns, and a new pattern different from those predicted for known vip1 genes. In the screening of vip2 genes, patterns similar to those of vip2Aa1, vip2Ba1/vip2Ba2, and vip2Ac1 genes were observed. The three predicted patterns for vip3Aa1, vip3Aa2, and vip3Af1 were found among vip3 genes, together with a new pattern indicating a novel vip3 gene. A tendency of *vip* and *cry* genes to occur together has been observed in this collection of *B. thuringiensis* strains.
A new family of insecticidal proteins secreted by *Bacillus thuringiensis* was discovered. The ability of *Bt* strains to produce a variety of insecticidal parasporal crystals (θ-endotoxins) has been described for decades. A class of vegetative insecticidal proteins was recently described: VIP 1/2 with activity against coleopteran pests and VIP 3 with activity against lepidopterans. However, the secreted proteins presented here bear no similarity to any of the previously described *Bt* proteins. In fact, no significant homology was found between the sequences of the here presented proteins and any of the thousands of protein sequences contained in the National Center for Genome Resources (GenBank, Santa Fe, NM). Homologs of the proteins presented here were identified by molecular and biochemical methods used in a high-through-put screen. The described proteins exhibit significant bioactivity towards coleopteran pests, specifically corn root worm and Colorado potato beetle.

**Antibacterial activity of *Bacillus thuringiensis* strains**

Galinia V. Kalmikova and Ljudmila I. Burtseva

Laboratory of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia

The antibacterial activity of 67 of *Bacillus thuringiensis* (Bt) strains of 43 subspecies against *Micrococcus lysodeiktics*, *M. candidus*, *M. luteus*, *M. flavus*, *M. varians* was analyzed. Only 8 Bt strains inhibited the growth of all tested strains of *Micrococcus* spp. The *Micrococcus* spp. strains were resistance to 23 Bt strains. Bt strains were examined for bacteriocin-like activity by a spot assay in double-layer agar plates. Bt spp. *thuringiensis*, *alesti*, *kurstaki*, *sumyoshiensis*, *fukaukensis*, *galleriae*, *entomocidus*, *darmstadtienis*, *dakota*, *shandongiensis*, *neoeleonis*, *mexicanensis*, *cameroon*, *leesis* strains demonstrated antibacterial activity against more than 50% of the tested Bt strains. In order to rule out a lytic activity of phages the method of Kekessy and Piquet was used. This method excludes direct contact between the producer and the indicator strains. The bacteriocin producing strains were immune to their own bacteriocins produced around colonies on solid media. However, two strains (morphovars of Bt spp. *kurstaki* and spp. *galleriae*) showed sensitivity to their own bacteriocins.

**Molecular cloning of novel crystal protein genes, cry30C and s2orf2, from a mosquitoicidal strain of *Bacillus thuringiensis* serovar *sotto* strain**

Akira Ohgushi,¹ Naoya Wasano,² Hiroyuki Saiioh,² Akiko Uemori,² and Michio Ohba

¹Graduate School of Agriculture, Kyushu University, Fukuoka, 812-8581, Japan, ²Biotechnology and Food Research Institute, Kurume, Fukuoka, 839-0861, Japan

Two novel cry genes, *cry30C* and *s2orf2*, were cloned from a mosquitoicidal strain of *Bacillus thuringiensis* serovar *sotto*. The *cry30C* and *s2orf2* genes encoded 77.4-kDa and 55.2-kDa proteins, respectively. The sequence of *Cry30C* possessed five conserved blocks commonly found in the existing Cry proteins, showing 60% identity to the *Cry30A* protein contained in a mosquitoicidal strain of *B. thuringiensis* serovar *medellin*. The *S2ORF2* protein had a high homology to that of the *S1ORF2* co-occurring in the same *sotto* strain. When the two genes were expressed in a *B. thuringiensis* *cry* mutant strain under the control of *cyt1Aa* promoter gene, the proteins were synthesized at high levels and accumulated as large inclusions. SDS-PAGE revealed that the inclusion is composed of two proteins of 72 and 55 kDa. Antibodies against the whole inclusion proteins of the *sotto* strain reacted with the 55-kDa protein only. The proteins of Cry30C and S2ORF2 exhibited no larvicidal activity against *A. aegypti*, and no cytotoxicity against HeLa cells.
factors in other pathogens, but their role in X. nematophila virulence has not been thoroughly investigated. X. nematophila produces two secreted proteases during in vitro growth, protease I (~100 kDa) and protease II (~55 kDa). Protease I is constitutively active, while protease II activity is only present in fractions collected during log phase growth. We have identified the gene in X. nematophila, prtA, which encodes protease II. In other pathogens prtA homologs encode secreted Zn2+ metalloproteases required for virulence. We have also identified a gene, prtX, necessary for transcription of prtA. The predicted PrtX sequence has similarity to part of a Mg2+ transport protein, MgtB from Salmonella enterica sv. Typhimurium. Both prtA::Tn5 and prtX::Tn5 strains show attenuated virulence towards Manduca sexta (tobacco hornworm) larvae, suggesting PrtA plays a role in killing the insect. In vivo targets of PrtA are currently being investigated. Study of the function and regulation of X. nematophila virulence factors, such as PrtA, may contribute to insect biological control methods.


An elastase found in early instar Lymantria dispar larval is involved in the proteolytic activation of Bacillus thuringiensis δ-endotoxins

Algimantas P. Valaitis

USDA Forest Service, Delaware, OH 43015, USA

A 50 kDa protease with elastase activity was purified from larval digestive fluids by a calcium precipitation step followed by ion-exchange and gel filtration chromatography. In contrast to trypsin and chymotrypsin, this elastase binds trypsin-activated Cry1A toxins. Stains blue with the cationic dye Stains-all, and is recognized by polyclonal antibodies raised to a putative Bacillus thuringiensis (Bt) toxin receptor, BTR-270. The digestion of Cry1A proteons using purified Lymantria dispar elastase generates an active toxin smaller than the product produced by either trypsin or chymotrypsin, implying that the elastase may be involved in the final step of the activation of Bt toxins, triggering the formation of a membrane-competent structure for insertion of the Bt toxin into the membrane and for toxicity.

Poster / Bacteria. B-10.

Influence of bacteriocin metabolites of Bacillus thuringiensis on antioxidants of gut of Galleria mellonella larvae

Elizaveta L. Dzhu, Natalia V. Redkina, Viktor V. Glupov

Laboratory of Insect Pathology, Institute of Systematic and Ecological of Animals, Russian Academy of Sciences, Frunze Street 11, Novosibirsk 630091, Russia

Bacillus thuringiensis ssp. galleriae (Bt) is entomopathogen sporeforming bacteria. As the result of their vital functions secondary metabolites isolate into the environment. δ-endotoxin exerts the most essential effect on insects. Bt isolates various proteins which have bactericidal characteristics. Development of bacterial infection is accompanied by destructive processes in midgut of larvae that results in rejection of oxygen-containing radicals and other toxic substances. In larvae’s midgut of Galleria mellonella changing of activity of enzymic antioxidants: superoxide dismutase (SOD), catalase (Cat), glutathione-S-transferase (GST), nonenzymic antioxidants: oxidized and reduce thiols (GSH/GSSG) were registered. The increase of activity SOD in 2 times on the 12th day and Cat on the 8th day and concentration of GSH / GSSG in 10 times on the 8th day under the influence of bacteriocin metabolites of Bt strains P-2 were found. The activity of GST lowered in 1.3 times on the 12th day, on the 16th day lowering of SOD activity in 2.25 times occurred. Changes in activity of antioxidants in midgut at the initial stages after feeding of bacteriocin metabolites are characteristic for acute toxicosis. The given changes in antioxidant system of G. mellonella larvae are characteristic for chronic diseases.

Poster / Bacteria. B-11.

Functional analysis of the cadherin protein from Heliothis virescens as Cry1Ac receptor

Juan L. Jurat-Fuentes,1,2 Michael J. Adang

Departments of Entomology and Biochemistry & Molecular Biology, University of Georgia, Athens, GA 30602, USA

Genetic knockout of the Heliothis virescens cadherin-like protein (HevCaLP) has been linked to high levels of resistance to Cry1Ac toxin (Gahan et al. 2001, Science 293:857-60). We demonstrated a lack of Cry1Aa binding to brush border membrane vesicles from larvae lacking HevCaLP (Jurat-Fuentes et al. 2004, Biochemistry 43:1429-305). Recently, HevCaLP peptide fragments expressed in Escherichia coli were reported to bind Cry1Ac in ligand blots (Xie et al. 2004, J. Biol. Chem. 280:8416-25). The goal of this work was to transiently express full length HevCaLP on the surface of insect cells to test its putative role as a Cry1Ac toxin receptor. We cloned the full-length cDNA encoding HevCaLP into the pTZ vector. Immunocytochemistry was used to detect HevCaLP expression on the surface of transfected Drosophila melanogaster S2 and Trichoplusia ni High Five cells. Cry1Ac toxin binding was tested using fluorescence microscopy, dot blotting and cell binding assays. The receptor role of HevCaLP was studied using fluorescence assisted cell sorting (FACS). In these assays, Cry1Ac bound to cells expressing HevCaLP. Therefore, while HevCaLP can be considered a functional receptor for Cry1Ac, the level of cytotoxicity may suggest the participation of additional molecules in Cry1Ac intoxication in vivo.

Poster / Bacteria. B-12.

CR12-MPED fragment of Manduca sexta Bt-Rα, cadherin enhances activity of Bt Cry1A toxins

Gang Hua,1 Jiang Chen,1 Juan Luis Jurat-Fuentes,1 Mohd Amir Abdullah1, Michael Adang2

Departments of Entomology1 and Biochemistry & Molecular Biology2, University of Georgia, Athens, GA 30602, USA

Cadherin-like proteins located in the midgut epithelium of lepidopteran larvae function as receptors for Bacillus thuringiensis Cry1 toxins. The cadherin protein Bt-Rα, from Manduca sexta larvae is a receptor for the Cry1A family of toxins (Hua et al. 2004. Insect Biochem. Molec. Biol. 34: 193-202). We also determined that cadherin-repeat 12 (CR12) and the membrane proximal extracellular domain (MPED) as critical regions in BtRα for Cry1Ac binding and cytotoxicity (Hua et al. 2004. J. Biol. Chem. 279: 28051-28056). The goal of this work was to identify radiolabeled CR12-MPED peptide produced in a heterologous system enhances the toxicity of Cry proteins towards target insects. Mixtures of CR12-MPED with Cry1Ab or Cry1Ac toxins fed to larvae increased Cry1Ac toxicity to M. sexta, Heliothis virescens and Helicoverpa zea significantly. Apparently, toxin binding was not necessary for the enhancing effect, since radiolabeled CR12-MPED bound Cry1Ab but not Cry1Ac. Histochemistry results demonstrated that the CR12-MPED peptide induced Cry1Ab but not Cry1Ac aggregation. Possible mechanisms leading to Cry1Ac toxicity enhancement by CR12-MPED will be discussed.


Mutagenic analysis of surface-exposed loop residues critical for larvicidal activity of the Bacillus thuringiensis Cry4Ba toxin

Tararat Khaokhiew and Chanon Angusathanosombat

Laboratory of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand

Previously, critical surface-exposed loop residues (P406 in β9-β10 loop, E417 in β6-β7 loop, Y465 and N466 in β6-β7 loop) in the receptor-binding domain of the Bacillus thuringiensis Cry4Ba toxin have been demonstrated to be involved in larvicidal activity. In this study, further mutagenic analysis was carried out to investigate a correlative
effect among these critical loop resides on Cry4Ba toxicity. Double mutants, P389A/E417A (β8-β9-β9-β10-β10 loops), E417A/Y455A and E417A/N456A (β8-β9-β9-β10-β10 loops) were constructed via PCR-based mutagenesis and highly expressed in Escherichia coli as 130-kDa protoxins in the form of inclusion bodies with yields comparable to the wild type toxin. When E. coli cells expressing each double mutant toxin was determined their toxicity against Aedes aegypti mosquito larvae, an almost complete lose in larvicidal activity was observed from all these double mutant toxins. In addition the double mutant toxins were structurally stable upon solubilisation and trypsin activation in carbonate buffer, pH 9.0. This results suggested that all these critical loop residues (P389, E417, Y455 and N456) are correlatively in larvicidal activity of the Cry4Ba toxin.

**Poster / Bacteria. B-14.**

**Studies of peptides mimicking the proposed pore-forming helices of the Bacillus thuringiensis Cry4Ba toxin**

Somphob Leechawee and Chanan Angsanthosombat

Laboratory of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakorn-Pathom 73170, Thailand

The α-helices 4 and 5 of 130-kDa Bacillus thuringiensis Cry4Ba toxin have been demonstrated to be important determinants of mosquito-larvicidal activity, particularly in pore formation. In this study, E. coli cells harboring the mutant plasmid-pS136NSSRN (T6) for the 130-kDa Cry4Ba mutant protoxin containing an additional proteolytic cleavage site in the loop between α4 and α5 were used for producing α4-α5 helical hairpins. The 130-kDa protein inclusion were solubilized in carbonate buffer, pH 9.0 and were activated by trypsin. The 65-kDa activated toxins were purified by the size-exclusion and further purified by reversed-phase HPLC using Jupiter 44106, USA

**Poster / Bacteria. B-15.**

**Isolation and functional characterization of Bacillus thuringiensis Cry4Ba toxin-binding proteins from Aedes aegypti larvae**

Seangdeun Moonsom and Chanan Angsanthosombat

Laboratory of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakorn-Pathom 73170, Thailand

Binding of Bacillus thuringiensis Cry toxins to susceptible larval midgut receptors results in toxin insertion and pore formation, leading to cell death by osmotic lysis. In this study, attempts were made to isolate and characterize Cry4Ba toxin-binding molecules. The 65-kDa FPLC-purified Cry4Ba mutant (R203Q/S204C) was covalently linked to the activated thiol sepharose 4B via position Cys-204 and used for affinity purification of a specific protein from CHAPS soluble fractions of Aedes aegypti homogenate. Binding interaction was performed in a binding buffer (0.3 M NaCl, 50 mM NaH2PO4, pH 8.0) containing 80 mM KI and bound toxin-gut complexes were eluted with the buffer containing 50 mM DTT. SDS-PAGE analysis via Coomassie brilliant blue staining revealed approximately 30-125 kDa larval gut proteins co-eluted with the purified Cry4Ba toxin. Binding specificity of these isolated proteins with the Cry4Ba toxin was confirmed by toxin overlay assays. Further characterization of these toxin bound proteins will be discussed.

**STU Poster / Bacteria. B-17.**

**Exposing the cryptic antibacterial potential of Cyt1Ca from Bacillus thuringiensis israelensis by genetic manipulations**

Mark Itsko1, Robert Manasherob2, Colin Berry1 and Arieh Zaritsky1

1Department of Life Sciences, Ben-Gurion University of the Negev, P.O.B. 653, Be’er-Sheva 84105, Israel, 2Department of Genetics Stanford University, School of Medicine Stanford, CA 94305-5120, USA

Cyt-like δ-endotoxins produced by Diptera-specific subspecies of Bacillus thuringiensis demonstrate cytolytic activity against a broad range of cells, bacteria included. These activities are mainly attributed to direct binding of the toxins to phospholipids. A recently discovered Cyt-like gene in B. thuringiensis subsp. israelensis, cyt1Ca was expressed in Escherichia coli. Its product Cyt1Ca was neither bacterial nor synergistic to Cry4A against Aedes aegypti larvae as does Cyt1Aa. The inactivity of Cyt-like domain of Cyt1Ca despite the high homology between them was addressed in this research, and related to its low hydrophobicity hence low binding ability to the cell membrane. Two approaches were undertaken to enhance hydrophobicity of this domain: (a) Site-directed mutagenesis to replace certain charged and polar amino acids in the Cyt-like domain of Cyt1Aa itself. The results acquiring, bactericidal abilities to the totally inactive Cyt1Ca is promising in clarifying the yet enigmatic general bactericidal effect of Cyt1Aa and dissecting it from the larvicidal effect.

**Poster / Bacteria. B-18.**

**Endogenic activation of Cyt2Ba toxin by camelysin from Bacillus thuringiensis israelensis**

Marina Nisnevitch1, Shmuel Cohen1,2, Eitan Ben-Dov2, Arieh Zaritsky2 and Rivka Cahani

1Department of Chemical Engineering and Biotechnology, College of Judea and Samaria, P.O.B. 3, Ariel 44837, Israel, 2Department of Life Sciences, Ben-Gurion University of the Negev, P.O.B. 653, Be’er-Sheva 84105, Israel

Bacillus thuringiensis israelensis is a Gram-positive spore forming bacterium that forms an insecticidal protein toxin crystal that is...
likely contributes the lowest amount of Cyt1A to the Bti parasporal
than BtiI or BtIII. However, the quantity of Cyt1A produced per unit
different from each other, with BtII producing much more per cell
expression of the BtI and BtIII promoter strains were significantly
promoters, but that BtIII is the weakest of the three, and therefore
the control. These results indicate that all three promoters are strong
amount of Cyt1A per spore. The levels and patterns of
analysis and correlated with spore counts and synthesis per unit
control Bti strain with the
YBT-1765 had been cloned and sequenced, which was the first
A 13kb replication region named ori165 of the large plasmid
indicated that the minireplicon (4.4kb) harbored three open reading

proteins, Cyt1A, Cry11A, Cry4A and Cry4B. Among the four
families: Cry and Cyt. The Cyt toxins include a main polypeptide,
composed of several toxin polypeptides which are divided into two
families: Cry and Cyt. The Cry toxins include a main polypeptide,
Cyt1Aa and two minor polypeptides Cyt2Ba and Cyt1Ca. Cyt2Ba
exists in its parasporal inclusions in a very low amount and can be
seen only by immunoblotting. Thereby we cloned cyt2Ba with the aid
of p20 gene in acrystalliferous BtiIPS78/11. The gene was highly
expressed as inclusion bodies which made it easy to obtain pure
Cyt2Ba protein. All known toxins of Cry family demand activation by
exogenous proteases cleaving peptide fragments on N- or C- termini
of the polypeptide. We found that solubilization of the toxin crystals
in the presence of spores and cell debris led to cleavage of Cyt2Ba
sequence between the 34 and 35 amino acid residues from
terminus. As was shown by SDS-PAGE, the endogenously activated
toxin had a molecular weight of 22kDa. The product of this cleavage
revealed haemolytic activity close to that of exogenously activated
Cyt2Ba species (by trypsin, chymotrypsin and proteinase K). The endoprotease
that caused the activation was identified as metalloprotease camelisin.

November Bacillus thuringiensis strains isolated from soil samples in
China.
Ying Meng, Zhenyu Zhang, Huidong Qu, Lifang Ruan, Ming Sun and
Ziniu Yu
College of Life Science and Technology, Huazhong Agricultural
University, State Key Laboratory of Agricultural Microbiology,
Wuhan 430070, P. R. China
Some novel Bacillus thuringiensis strains were isolated and screened
from soil samples in China based on its crystal protein profiles and
the shapes of parasporal crystals. N-terminal 15 amino acid sequences
were detected for the crystal proteins. Five of them showed no
significant similarity with known Bacillus thuringiensis crystal
proteins. Three of them share high homologous with S-layer proteins
in Bacillus cereus group. For those strains with novel N-terminal
amino acid sequences, the toxicity targets include Lepidoptera
insects, Diptera insects, and root-knot nematodes. Partial nucleotide
sequence of crystal protein gene from YBT-987 showed N-terminal
157 amino acid sequence is most close to that of Cry8Ba proteins
(33% identity), suggesting that this strain may harbor a Class I novel
crystal protein gene.

Diversity of Bacillus thuringiensis strains in the maize and bean
phyloplane and from their respective soils in Colombia
Silvia Jara1, Pau Maduell1,2, Sergio Orduz1,3
1Unidad de Control Biológico y Biotecnología, Corporación para
Investigaciones Biológicas (CIB). Medellín, Colombia, 2Unidad de
Microbiología, Facultad de Ciencias, Universitat Autònoma de
Barcelona, Barcelona, España, 3Facultad de Ciencias Básicas,
Universidad de Pamplona, Pamplona, Colombia.
Bacillus thuringiensis was isolated from the phylloplane and soil of
maize and bean plants from three localities in Antioquia, Colombia.
Ninety six samples from the phylloplane and 24 from soil were
analyzed. 214 isolates were obtained from the phylloplane samples,
while 59 isolates were recovered from 24 soil samples. Sixty five per
cent and twelve percent from the phylloplane and soil isolates,
respectively, showed activity against Spodoptera frugiperda. These
isolates contained proteins of 130 and 70 kDa, similar to that of
B. thuringiensis serovar. kurstaki. Isolates 147-5406, 147-5402, 147-
5501, 147-5401, 147-5502 and 147-5404, exhibited high toxic
activity against Spodoptera frugiperda, and all had the genotype
cry1Aa, cry1Ac, cry1B, and cry1D, the most abundant genotype
found in the study. In contrast, 27% of the phylloplane isolates and
88% of the soil isolates were active against Culex quinquefasciatus
and had protein profiles similar to B. thuringiensis serovar. medellin
and B. thuringiensis serovar. israelensis. The predominant population of
B. thuringiensis on the phylloplane harbored cry1 genes and was
active against S. frugiperda whereas in soil, isolates harboring cry11
and active against Culex quinquefasciatus predominated.
Characterization of selected Bacillus thuringiensis strains
Galina V. Kalmykova1, Ljudmila I. Burtseva1, Anna V. Mokeeva2, Svetlana F. Oreshkova2
1Laboratory of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia; 2Vector State Research Center of Virology and Biotechnology, Institute of Bioengineering, Kol'tsovo, Novosibirskaya oblast, Russia

The characterization of 60 Bacillus thuringiensis (Bt) strains of 27 subspecies was based on scanning electron microscopy, PCR analysis using general primers for cry1, cry2, cry3, cry4, cry7,8, cry11A and their insecticidal activity against Gryllus bimaculatus (Orthoptera), Aedes aegypti (Diptera), Galleria mellonella, Pyurausta sticticalis, Lymantria dispar (Lepidoptera). Microscopic observations of these strains revealed that crystal shapes and sizes were variable. Of 60 tested strains, 23 produced large bipyramidal crystals (as large as 2.5 micrometers), and were toxic to Lepidoptera, however, 16 of them harbored cry1, cry2 and were toxic to Lepidoptera and Diptera. Moreover acrystalliferous variants of Bt ssp. thuringiensis, galleriae and Bt ssp. kurstaki, yunannensis reacted with cry1 and cry1, cry2 general primers, correspondingly. Nevertheless they were not toxic to tested insects. Bt ssp. soto, israelensis, dacota, indiana, kumamotoensis, tochiensis, mexicana, monterrey appeared small bipyramidal crystals harbored cry 7.8 and were nontoxic. Strains of Bt ssp. israelensis and ssp. morrisoni (PG-14) had large irregularly shaped crystals, harbored cry4, cry11 and were toxic to Diptera. Bt ssp. darmstadensis strain with small irregularly shaped crystals, harbored cry4 and was toxic to Diptera. None of 60 strains was positive for cry3, and 18 strains didn’t react with all tested primers.

Effects of Bacillus thuringiensis var. kurstaki toxins on Scheloribates praeincisus (Berlese, 1910) (Acar: Oribatida: Scheloribitae)
Aníbal R. Oliveira, Italo Delalibera Júnior and Thiago R. Castro
Department of Entomology, Plant Pathology and Agricultural Zoology, ESALQ – University of São Paulo, C.P.9, 13418-900, Piracicaba-SP, Brazil

Dipel is a formulation of Bacillus thuringiensis var. kurstaki highly toxic to lepidopterans. Although some effects of Bt-toxins on mites of the suborders Astigmata, Mesostigmata and Prostigmata have been reported, effects on mites of the suborder Oribatida are unknown. Oribatid mites are one of the most abundant and diverse groups of organisms acting upon food chains responsible for the decomposition of organic matter. They are a group of non-target organisms most exposed to the Bt-toxins released in the soil. In this study, we evaluated the effect of ingestion of Dipel WP by Scheloribates praeincisus, a common oribatid in Brazil. Mites were fed on macerated cotton leaves with and without Dipel (0.1 mg/mg of dry leaves). There was no detectable effect of Dipel on survival of adults and immatures and on immature developmental time.

Effect of Bacillus thuringiensis strains on Spodoptera cosmioides
Pedro J. Neves1, Karen B. Santos1, Ana M. Menegumi1, Gislane T. Vilas-Bôas1, Walter J. Santos1, Olivia M.N. Arantes1
1Universidade Estadual de Londrina-UEL, Londrina-PR, Brazil, 2Instituto Agronômico do Paraná -IAPAR, Brazil

Spodoptera cosmioides has occurred at increasingly higher numbers in cotton fields, causing significant damage. Frequent broad spectrum insecticide applications are required to control this pest. Due to the direct and indirect effects of agrochemicals on human health and on the environment, new pest control strategies have been developed. Hence, the objective of this work was to evaluate the pathogenicity of Bacillus thuringiensis strains on 2nd instar Spodoptera cosmioides. Anticarsia gemmatalis artificial diet cubes (1 cm3) were dipped into a bacterial solution at the concentration of 108 spores per mL and offered to caterpillars placed in 20-well cell plates. Forty caterpillars were used in each treatment. The experiment was conducted in B.O.D. chambers at 25±2°C, 60±10% RH, and a 14h photophase. Mortality assessment was performed after 72h. The mortality of caterpillars exposed to strains Br36, Br37, Br45, Br7, Br10, and Br9 was 66%, 95%, 86%, 87%, 73%, and 100% respectively, demonstrating a high efficacy against this pest. This study is part of a larger project aimed at evaluating the biological activity of Bacillus thuringiensis against the species Spodoptera eridania, Spodoptera frugiperda, and Spodoptera cosmioides, which cause serious problems in cotton in Brazil.

Study on preparation of Bacillus thuringiensis controlling both Lepidoptera and Coleoptera pest
Ping Cheng1, Ming Sun2, Ziniu Yu3, Shouwen Chen3, Heshan Xie1, Guohui Yu4
1Zhuhai Agricultural Science Research Centre, Zhuhai, Guangdong, 519075, People’s Republic of China, and 2Department of Microbial Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, 430070, People’s Republic of China

Recombined plasmid pMBM305-06R was constructed by cry3A gene coding Coleoptera-specific protein, and then transformed into a wild B.t. strain YBT803-1 by electroporation. A transformant MBMY-001 was obtained. The stabilizer, antiseptic and emulsifier for suspending agent was systematically researched, also the stabilizing and accessory ingredient for wettability pulvis was studied and found the best stabilizing (D-5) and accessory ingredient (S-1). A new bioassay method was set up for genetic engineering biotic insecticide, and results showed that MBMY-001 was not only highly toxic to Phylodecta vulgarisitima larva (LC50 0.41±3µL/mL) but also toxic to Plutella xylostella (LC50 3.31±3µL/mL), but the virulence to P. xylostella was only 1/3 of wild strain YBT803-1. Field experiment showed high control efficiency for P. xylostella (92.47%) and Phyllotreta striolata (82.5%), and no obvious influence on the natural enemy.

Molecular dynamics simulation of Bacillus thuringiensis Cry4a mosquito-larvicidal protein in explicit water
Taveechai Taveecharoenkod1, Teerakiat Kerdcharoen2 and Chanan Angsuthanasombat1
1Department of Immunology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand; 2Department of Physics and Capability Building Center for Nanoscience and Nanotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand, 1Laboratory of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand

To gain knowledge of structural dynamics and properties of mosquito-larvicidal protein Cry4Aa in aqueous solution, molecular dynamics simulation was employed to investigate structural dynamics, properties and the influence of water molecules on this protein. By calculating distances between the center of mass of each of the Cry4Aa three domains in 10 nanosecond, it showed gradual separation of domain II from domain III, and gradual moving of domain I to domain II. However, domain I of the closely related toxin, Cry4Ba, exhibited gradual separation from the two other domains. In addition, a difference in dynamics was observed between Cry4Aa and Cry4Ba in aqueous solution. When root-mean-square of position displacement (rmsd) and a number of hydrogen bonds as a function of time of Cry4Aa in aqueous solution were analysed, it revealed a high rmsd observed in Cry4Aa, suggesting that a flexibility in the structure is required for conformational changes in prior to membrane insertion and pore formation in the cell membranes. The number of hydrogen bonding between protein-water molecules and water-water molecules, together with solvent accessible surface area (sasa) of both hydrophobic and hydrophilic parts indicated a significant decrease in sasa and a relative increase in the system hydrophobicity as Cry4Aa was simulated in aqueous solution.
The subterranean burrower bug, *Cyrtoctenomus bergi* Froeschner, is a polyphagous insect pest in different tropical areas. *C. bergi* is reported as a pest of cassava, potatoes, onions, peanuts, maize, sorghum, coffee, sugarcane, and pastures. Little is known about natural antagonists of this pest insect.

**Impact of Bacillus thuringiensis Cry toxins on the predator *Euseius concordis** (Acarina: Phytoseiidae)

Fernando H. Ibañes and Italo Delalibera Júnior

Department of Entomology, Plant Pathology and Agricultural Zoology, ESALQ - University of São Paulo, C.P.9, 13418-900, Piracicaba–SP, Brazil

Phytoseid mites are the most important predators of phytophagous mites. Many species of predatory mites feed on spider mites as well as pollens and plant exudates. Due to the external application of *Bacillus thuringiensis* (Bt) based products, cell content feeders such as spider mites, and their predators do not ingest considerable amount of Bt and hence side-effects on these non-target are not expected. The situation is different when predatory mites feed on pollen, plant exudates from Bt plants or spider mites reared on Bt plants because they may also ingest Bt toxins. The information of side effects of Bt toxins on phytoseid mites is scarce. In this study, we developed a method to assess adverse effects of Bt toxins on phytoseid mites. The method consisted on stimulating the mites to ingest the test solution by maintaining them in a relatively dry chamber (55-65% RH). The solution tested contained a blue dye (food color additive) to confirm ingestion. This method was validated using the Bt product Dipel WP (0,005 g/ml) on the predatory mite *Euseius concordis*. Ingestion of Dipel decreased adult longevity and oviposition of *E. concordis* compared to the control.

**Light and electron microscope investigations on a rickettsial disease of the subterranean burrower bug, *Cyrtoptenomus bergi* Froeschner (Hemiptera: Cydnidae)**

Regina G. Kiesbye

Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, 64287 Darmstadt, Germany

Light and electron microscope studies revealed that the death of this insect is caused by a rickettsial disease. Histological sections showed that the fat body is heavily infected and hypertrophied. The oval-shaped rickettsial bodies measure 0.5 x 0.26 µm in size and are located in the cytoplasm of the fat body cells. No associated crystals could be observed. Genetic investigations are planned for closer determination of this rickettsial species that could be of interest in view of biological control measures against *C. bergi*.

**Effects of Bacillus thuringiensis on the predatory mite *Euseius concordis*** (Acar: Phytoseiidae)

Oscar E. Guevara1, Abraham Bulles1 and Sergio Orduz2

1Unidad de Biotecnología y Control Biológico, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia. 2Facultad de Ciencias Básicas, Universidad de Pamplona, Pamplona, Colombia

In order to study the possible emergence of resistance, a wild colony of *Aedes aegypti* was subjected to selection pressure with the *B. thuringiensis* serovar. *israelensis* Cry11Aa toxin. This bacterium is the base of the most important biopesticide used in the control of mosquito vectors all over the world. After 33 generations of selection, no significant resistance levels were obtained. Selection experiments started with the Cry11Aa half lethal concentration (LC50) of 26.3 ng/ml and at the generation 33 the LC50 was 84.6 ng/ml. The highest rate of resistance (RR) found was 3.05, detected when the LC50 between treated and untreated colonies were compared in generation 33. Kinetic mortality experiments performed with 500 times the Cry11Aa LC50 indicate that the susceptible population died faster than the treated one, and 100% of larval mortality was reached within 330 minutes, while at this same time, 30% of the treated population remained alive. These data indicate that the development of resistance in *A. aegypti* to the *B. thuringiensis* serovar. *israelensis* Cry11Aa toxin might take longer time than in *Culex quinquefasciatus*. 

**Biology and nutrition of resistant and susceptible populations of *Anticarsia gemmatalis*** (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*

Flavio Moscardi1, Andrea B. Malaguido2 and Clara B. Hoffmann Campo1

1Embrapa Soja, C. Postal 231, 86001-970, Londrina, PR, Brazil, 2Embrapa-Soja/Pronex, Londrina-PR, Brazil

A colony of *Anticarsia gemmatalis* was selected in the laboratory for resistance to *Bacillus thuringiensis* (Bt), through continuous selection pressure by a commercial formulation of Bt (Dipel). This population had a resistance ratio of about 50-fold to Bt, at the time it was compared with the selected (susceptible) population, regarding biological, nutritional and physiological parameters. Evaluation of larval development and survival, pupal weight and survival, sex ratio, adult reproductive capacity and longevity were based on an initial number of 80 larvae/treatment. For the evaluation of nutritional and physiological parameters, 40 larvae were used plus 20 larvae used as alquots for the different determinations for each treatment. The main differences between resistant (RP) and susceptible (SP) populations of *A. gemmatalis* occurred for some of the biological parameters evaluated. Mean total larval development time was significantly higher in the RP than in the SP larvae. The sexual ratio of emerged adults (female/male) was 1.34 for SP compared to 0.82 for RP. Mean peak oviposition of the SP occurred in the 5th day (ca. 124 eggs/female) after female emergence, while peak oviposition for the RP occurred in the 6th day (ca. 85 eggs/female) after female emergence. Other biological parameters evaluated were not significantly affected by the treatments. In general the observed biological differences between the RP and the SP could not be explained by the nutritional and physiological parameters evaluated.
A common, but complex, mode of resistance of *Plutella xylostella* to *Bacillus thuringiensis* toxins Cry1Ab and Cry1Ac

**Sales Ibiza-Palacios** 1, Ali H. Sayyed1, Roxani Gatsi2, Denis J. Wright3, Neil Crickmore1 and Baltasar Escriche1

1Departamento de Genética, Universitat de València, Dr. Moliner 50, 46100 Burjassot (Valencia), Spain. 2Department of Entomology, Faculty of Life Sciences, Imperial College London, Silwood Park campus, Ascot, Berkshire SL5 7PY, UK. and 3School of Life Sciences, University of Sussex, Falmer, Brighton, East Sussex BN1 9QG, UK

A field population of *Plutella xylostella* from Malaysia (SERD4) was selected in laboratory with *Bacillus thuringiensis* toxins Cry1Ab and Cry1Ac. The Cry1Ac-SEL population showed a little cross-resistance to Cry1A, Cry1Ca and Cry1Da with incomplete mode of inheritance of resistance to Cry1Ac. The Cry1Ac-SEL population showed a marked cross-resistance to Cry1A (40-fold; Sayyed and Wright, 2001). In the present studies resistance to Cry1Ab was characterised both by genetic and biochemical approaches. Mode of inheritance of resistance to Cry1Ab was examined in Cry1Ab-SEL SERD4 by standard reciprocal crosses using laboratory susceptible population (ROTH). Logit regression analysis of F1 reciprocal crosses indicated that resistance to Cry1Ab was inherited as incompletely dominant trait. Binding studies showed a large reduction of specific binding of Cry1Ac and Cry1Ab to midgut membrane vesicles of the Cry1Ab-SEL sub-population. The resistance phenotype in both sub-populations Cry1Ab-SEL and Cry1Ac-SEL could be partially overcome by challenging the selected populations with trypsin-activated toxins rather than native protoxin although no defect in toxin activation could be identified. Present and previous results indicate a common but complex basis of resistance to both Cry1Ab and Cry1Ac selected sub-populations.

**Lack of binding of Bacillus thuringiensis Cry1A toxins as the basis of resistance in a greenhouse-derived population of Trichoplusia ni**

Ana Rodrigo-Simón1, Ping Wang2, Jian-zhou Zhao2, Anthony Shelton2 and Juan Ferré3

1Departamento de Genética, Universidad de Valencia. Dr. Moliner 50, 46100 Burjassot (Valencia), Spain. 2Department of Entomology, Cornell University, Geneva, NY 14456, USA

Field-derived resistance to *Bacillus thuringiensis* (Bt) toxins, so far only found in *Plutella xylostella* populations, has been characterized by lack of binding of Cry1A toxins to the larval midgut. Resistance to Bt var. *kurstaki* in *Trichoplusia ni* populations has been recently reported in Canadian commercial greenhouses. One of these populations, with originally a moderate level of resistance, was re-selected in the laboratory with Cry1A to midgut resistance to this toxin increased to around 1000-fold. *In vitro* binding assays with iodinated Cry1Ac and Cry1Ab, and BBMV from *T. ni* showed no binding in the resistant strain. As expected, the susceptible and the F1 progeny bound both toxins specifically, and the global affinity of Cry1Ac was slightly higher in susceptible than in F1 insects. It was previously shown that Cry1Ab and Cry1Ac compete for the same binding site in this insect. Our results show that *T. ni* can become resistant to the above two toxins by altering the shared site. Therefore, to prevent the development of resistance in *T. ni*, Cry1Ab and Cry1Ac should not be combined in the same transgenic plant and, seemingly, Bt strains used in commercial formulations should contain, besides Cry1Ab or Cry1Ac, other insecticidal toxins effective against *T. ni*.

**Comparative analysis of Bt toxins binding among susceptible and resistant strains of European corn borer**

Joel González-Cabrera1, Herbert A. Siqueira2, Blair D. Siegfried2, and Juan Ferré3

1Departamento de Genética, Facultad de CC. Biológicas, Universidad de València, 46100-Burjassot, Valencia, Spain. 2Department of Entomology, 202 Plant Industry Bldg, University of Nebraska-Lincoln, NE 68583, USA

The European corn borer (ECB) is one of the most economically important pests worldwide. Bt-corn expressing Cry1Ab toxin from *Bacillus thuringiensis* (Bt) seems to be the best alternative to chemical pesticides in controlling this pest. However, this technology will not be successful if resistance develops in pest populations. The alteration of binding to midgut receptors is the best characterized mechanism of resistance to Bt toxins. We have tested one susceptible and two resistant ECB strains that were obtained after selection in the laboratory with full-length Cry1Ab toxin. Resistant strains showed at least 100-fold resistance to Cry1Ab, high cross-resistance to Cry1Ac and a very low level to Cry1Fa. Moreover, resistance seems to be polygenic in both strains. Here, we tested if binding site alteration is the mechanism of resistance in these two strains. Labeled Cry1Ab and Cry1Ac bound specifically to BBMV from susceptible and both resistant strains. Although previous studies have indicated differences in Cry1Ab binding to BBMV proteins, competition experiments showed that binding parameters were similar among strains. Furthermore, analysis of binding stability showed no significant differences among strains. Overall, our results do not support that resistance in the resistant strains is associated with alteration in binding to toxin receptors.
(~100-fold). Selection with Cry1Ac for six generations increased resistance >150-fold, it also gave a significant level of cross-resistance to deltamethrin (1000-fold). Synergist studies indicated that resistance to deltamethrin and Cry1Ac in selected sub-populations could be esterase associated. Initial studies using BBMV prepared from both selected sub-populations suggest that esterases have a strong affinity for the Bt toxin Cry1A.

**STU Poster / Bacteria. B-38.**

**Analysis of midgut proteinases from Bacillus thuringiensis susceptible and resistant Heliothis virescens**

(Lepidoptera: Noctuidae)

Lohitash Karumbaiah1, Brenda Oppert1, Juan L. Jurat-Fuentes1, Michael J. Adang1,2

Departments of 1Entomology, 2Biochemistry and Molecular Biology, University of Georgia, Athens, GA-30602, 2USDA ARS Grain Marketing and Production Research Center, Manhattan, KS-66502, USA

_Heliothis virescens_ is a major lepidopteran pest of cotton in the United States and the target insect of _Bacillus thuringiensis_ (Bt) transgenic cotton. We conducted an analysis of gut proteinases from Bt susceptible and resistant _H. virescens_ strains, including the susceptible strain, YDK, and three resistant strains, YHD2-B, CXC, and KCBhyb. Casein zymogram analysis of YDK and YHD2-B gut extracts did not reveal significant differences. However, two unique bands of caseinolytic activity were observed in CXC and KCBhyb. Kinetic microplate assays with a trypsin substrate demonstrated that proteinases in YDK gut extract had more alkaline pH optima compared to YHD2-B, CXC and KCBhyb. In assays with a chymotrypsin substrate, enzymes in YDK extracts had lower alkaline pH optima in contrast to those in YHD2-B gut extract. Enzymes from KCBhyb gut extracts had the highest activity of all strains in alkaline buffers, particularly at pH 10.6, similar to the physiological pH of the lepidopteran midgut. Temporal Cry1Ac protoxin activation indicated that YHD2-B gut extract processed protoxin at a slower rate than that of YDK. Because gut proteinases are a critical component of Bt toxin mode of action, these differences may contribute to decreased toxicity in the Bt-resistant strains.

**Poster / Bacteria. B-39.**

**Mixing and matching of toxin complex proteins**

Timothy Hey, Scott Bevan, Amanda Schleper, Patricia Birkhold, Stephanie Burton, Tom Meade, Don Merlo, Joel Sheets, Robin Thompson and Haley Moon

Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268, USA

Bacteria in the genera _Photorhabdus_ and _Xenorhabdus_ produce several classes of _Toxin Complex_ proteins. The _Class A_ proteins (~280 kDa) possess insecticidal activity. The _Class B_ (170 kDa) and _Class C_ (~110 kDa) possess no apparent insecticidal activity. Several laboratories have demonstrated that potent insecticidal activity requires all three classes of protein (A, B and C) with the _Class B_ and _Class C_ proteins potentiating the activity of the _Class A_ proteins by as much as 1000 fold. Toxin Complex has been considered as a possible candidate for use in transgenic plants, especially since Bt resistant insects are susceptible to Toxin Complex. We have designed novel _Toxin Complex_ gene constructions to increase plant transformation efficiency and to provide coordinated expression of three genes in plants.

**Poster / Bacteria. B-41.**

**Cloning and expression in a methylotrophic bacterium of an insecticidal crystal protein from Bacillus thuringiensis**

Larry Gringerter1, Young Choi1, Lyne Morel2, Luke Masson2 and Carlos Miguez2

1Great Lakes Forestry Centre, Canadian Forest Service, 1219 Queen St. E., Sault Ste. Marie, Ontario, P6A 2E5, Canada, 2Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Ave., Montreal, Quebec H4P 2R2, Canada

Methylotrophic bacteria are capable of growth in a completely synthetic medium consisting only of mineral salts and single-carbon compounds, such as methanol or methylamine, as the sole carbon and energy source. These simple and relatively inexpensive requirements render large-scale fermentations of methylotrophs very cost-effective. This feature, along with the availability of genetic tools and abundant genetic and physiological information on methylotrophic bacteria, makes this group of microorganisms very attractive economically as potential hosts for the mass production of recombinant proteins. In addition, their often ubiquitous presence in nature and reports of possible endophytic relationships with trees and plants suggests that perhaps certain species could also serve as an environmentally safe and effective delivery system of recombinant biocontrol agents, such as _Bacillus thuringiensis_ delta-endotoxins, against crop and forest insect pests. To this end, we cloned and expressed the _cry1Aa_ gene from _B. thuringiensis_ in the methylotroph _Methylobacterium extorquens_ and began an investigation of its properties. Transmission electron microscopy revealed characteristic bipyridential, intracellular delta-endotoxin crystals. In single dose assays of the recombinant (140 µg dry weight) against the silkworm, _Bombyx mori_, both whole cells and cell lysates caused immediate feeding inhibition followed by 100% mortality.

**SYMPOSIUM (Div. of Nematodes). Wednesday, 8:00-10:00**

**Genomics of entomopathogenic nematodes and symbiotic bacteria**

Syposium. Wednesday, 8:00. 89

_The Xenorhabdus genome project_  
Steven Forst

Department of Biological Sciences University of Wisconsin Milwaukee, WI 53201

_Xenorhabdus_ spp. are insect pathogenic bacteria vectored by their symbiotic entomopathogenic nematode partners. The role of the flagella regulon in symbiosis and pathogenesis of _Xenorhabdus_ is currently not well understood. The FlhDC master flagellar regulator is involved not only in flagellar production but also in virulence and hemolysin and lipase production. Recent studies have shown that motility, virulence and exoenzyme production are controlled by an integrated network of regulation. Genomic analysis of the flagella regulons in _Xenorhabdus nematophila_ and _Xenorhabdus bovienii_ has revealed that while the basic flagellar gene clusters are conserved many nonflagellar genes located within the flagella regulons are
widely divergent. The significance of these findings in the unique life cycle of *X. nematophila* will be discussed.

**Symposium. Wednesday, 8:20.**

*Photorhabdus*: Functional genomics of an insect pathogen

N. Waterfield, A. Dowling, M. Hares, R. ffrench-Constant

University of Bath, UK

We have been mining the genome of *Photorhabdus* for novel pesticides and drugs. We will present a range of sequence and screening based approaches for the isolation of novel insecticidal proteins and protein antibiotics. Work has focused on three classes of insecticidal proteins. The Toxin complexes which confer oral toxicity to caterpillar pests. The Mcf toxins which are injectably active and induce apoptosis in both insect and mammalian cells. The PirAB binary toxins which are toxic to mosquito larvae when expressed separately and then combined. Progress on functional expression of these toxins will be presented and an update given on the development of Tc expressing transgenic plants.

**Symposium. Wednesday, 8:40.**

*Nutrition and signal exchange between Photorhabdus and its invertebrate hosts*

Robert J. Watson, Jane Williams, Marie Thomas, Georgette V. Spencer, Susan A. Joyce and David J. Clarke

Molecular Microbiology Laboratory, Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK

*Photorhabdus* is a genus of entomopathogenic, gram-negative bacteria normally found colonising the gut of a specialised stage of the nematode Heterorhabditis called the infective juvenile (IJ). The IJ is a free-living stage of the nematode that seeks out and infects insect larvae. The *Photorhabdus* are released from the IJ into the insect hemolymph where the bacteria rapidly grow and the IJ dies 48-72h after the initial infection. The nematode then grows and reproduces in the insect cadaver by feeding on the *Photorhabdus* bacterial biomass. After several generations the nematodes develop into IJs and the *Photorhabdus* bacteria re-colonise the nematode gut. During this life cycle the nematode relies on the bacteria for nutrients and in this study we show that iron acquisition by *Photorhabdus* plays a key role during the course of the tripartite bacteria-nematode-insect interaction. Therefore, during growth in the insect, *Photorhabdus* must accumulate sufficient iron from the insect tissues to satisfy the nutritional requirements of the nematodes. In addition, we present evidence suggesting that the stilbene antibiotic produced by *Photorhabdus* may also serve as a signal to control nematode development. Therefore, as is the case in other mutualistic relationships, the interaction between *Photorhabdus* and its nematode host involves the exchange of nutrients and signals.

**Symposium. Wednesday, 9:00.**

*Heterorhabditis bacteriophora* genome sequence: A glimpse into the first 1000 expressed sequence tags

Parwinder Grewal

Department of Entomology, The Ohio State University, Wooster, OH 44691, USA

A total of 1246 expressed sequence tags (ESTs) were generated by random sequencing of clones from a cDNA library of the infective juvenile stage of the entomopathogenic nematode, *Heterorhabditis bacteriophora*. Approximately 453 of the 1072 acceptable ESTs (42%) had significant similarities to the annotated sequences in GenBank but 643 (58%) did not find any significant similarity to existing databases. About 19% of the annotated ESTs belonged to the genetic information processing, 15% to metabolism, and 14% to the environmental information processing, all three important pathways to the functioning of the infective juveniles. Several ESTs were similar to genes that have role in aging (akt-1, pdk-1 & daf-7), stress resistance genes such as superoxide dismutase (sod-4), heat shock genes (hsp-70), eat genes, and signaling proteins like G-protein coupled receptors, regulators of G-protein signaling (rgs), and serine/threonine kinases. ESTs also included sequences with similarities to putative virulence factors, such as protease inhibitors, cysteine proteases (cathepsin-B and -L-like) and serine protease. Another useful match was to an active TcA transposable element, which can prove to be a useful tool in functional genomics of entomopathogenic nematodes.

**Symposium. Wednesday, 9:20.**

Developing tools of genetics and genomics in *Heterorhabditis bacteriophora*

András Fodor, Parwinder S. Grewal and Michael G. Klein

Department of Entomology, Ohio State University, Wooster OH 44691, USA

The genomic sequence of the entomopathogenic nematode Heterorhabditis bacteriophora is on the way. The biological meaning will be elucidated by functional genomics based on available sequence information, and the genetics of the organism. We elaborated a system for getting reproducible results of genetic and functional genomic analyses in *H. bacteriophora*. We established: (i) a solid media (ENGM) on which *H. bacteriophora* could be propagated and handled individually; (ii) mutants (NS107, HU1956) of *Photorhabdus luminescens* TT01, which do not overgrow the nematodes; (iii) inbred lines of TT01 and GP11 nematodes; (iv) a transformation protocol for *P. luminescens* TT01 and transformed our mutants with color markers; (v) a system for studying RNAi activity of C. elegans genes cloned in pl4440. We induced dpy-3 RNAi phenocopies in *H. bacteriophora* by feeding. We work on isolating mec-1 mutant of *P. luminescens* TT01 HU1956 and NS107 strains to increase the frequency of RNAi by using mec(-) mutants. Primers were designed for the Tc 1 transposase of C. elegans.

**Symposium. Wednesday, 9:40.**

Application of forward and reverse genetics for the study of symbiosis in an entomopathogenic nematode host

Todd A. Ciche 1, 2 and Paul W. Sternberg 1

1Howard Hughes Medical Institute and The Biology Division, California Institute of Technology, Pasadena CA, 91106, USA,
2Current address: Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA

The nematode Heterorhabditis bacteriophora is both an insect parasite and a host in an obligate mutualism with the insect pathogenic bacterium, *Photorhabdus luminescens*. The tripartite interactions between nematode, bacterium and insect involves several interesting attributes e.g.; 1) symbiotic specific colonization of the intestinal lumen of the infective juvenile (IJ) stage nematode; 2) hunting, infection and regurgitation of *P. luminescens* bacteria by IJs in hemolymph, and 3) symbiont dependent growth and reproduction of the nematode. Although *H. bacteriophora* and Caenorhabditis elegans are both in the family Rhabditidae, genetic techniques were underdeveloped and not applied for the study of symbiosis. Here we show the successful application of RNAi in *H. bacteriophora*. First larval stage nematodes were soaked with *in vitro* transcribed dsRNA corresponding to *H. bacteriophora* orthologs of *cct-2* (HS6P60 chaperonin), *daf-21* (*HS900*, icd-1 (anti-apoptotic BTF3 transcription factor), 2 ribosomal biogenesis genes (W01G7.3, W01B11.3) and a guanine nucleotide binding protein (*K04D7.1*) which had strong phenocopies in C. elegans. For *H. bacteriophora*, the penetrance of RNAi was high (70-100%) except for *K04D7.1* that had moderate penetrance (~50%). The resulting phenocopies were similar but not identical in *H. bacteriophora* and C. elegans. Forward genetics using the mutagen EMS was moderately successful in *H. bacteriophora* resulting in 2 alleles corresponding to “unc-22” or “twitchin” and several egl (egg laying defective) and dpy (dumpy) phenotypes. We are applying forward and reverse genetics to elucidate host genes involved in symbiotic specific colonization of the nematode gut and growth and development as well as insect parasitism.
A molecular diagnostic method for selected Ascosphaera species using PCR amplification of internal transcribed spacer regions of rDNA

K. Daniel Murray1, Katherine A. Aronstein2, and Walker A. Jones3

1Texas A&M Agricultural Experiment Station, Weslaco, TX, USA. Current address: USDA-ARS, Honey Bee Research Unit, Kika de la Garza Subtropical Agricultural Center, Weslaco, TX 78596, USA,
2USDA-ARS, Honey Bee Research Unit, Kika de la Garza Subtropical Agricultural Center, Weslaco, TX 78596, USA, 3USDA-ARS, Beneficial Insects Research Unit, Weslaco, TX, USA. Current address: USDA-ARS, European Biological Control Laboratory, France

Ascosphaera spp. fungi are associated with social and solitary bees, in some cases as pathogens causing chalkbrood disease. As a supplement to morphological identification, we developed a simple PCR-based identification method for selected Ascosphaera species. We exploited sequence differences in the internal transcribed spacer regions of rDNA to design species-specific primers. Analysis involves simply scoring the presence or absence of a single band for a given pair of primers. The method can distinguish the four Ascosphaera species known to be associated with honeybees. It also distinguishes Ascosphaera aggregata, the chalkbrood pathogen of the alfalfa leafcutting bee, from another Ascosphaera species associated with this bee. We expect the method will be useful for identifying and determining purity of Ascosphaera cultures, and may be a first step toward development of an early detection method of chalkbrood infection in honeybees and leafcutting bees.

The entomopathogenic fungus, Beauveria bassiana displays varying degrees of virulence towards several tick species including Ixodes scapularis, Rhipicephalus sanguineus, Amblyomma maculatum, A. americanum, and Dermacentor variabilis. The latter two species display a certain level of resistance to fungal infection that can be partially overcome by defined inoculum conditions. These conditions appear to be linked to the production of oxalic acid by the fungus. Several lines of experimental evidence support the hypothesis that oxalic acid secretion by B. bassiana coupled to a reduction in the pH of the medium, act as potent acaracidal factors during pathogenesis. (1) Acaracidal activity of culture supernatants was retained after treatments including boiling and protease digestion, but was lost after dialysis. (2) Metabolite analysis indicated oxalate to be the major secreted organic compound present in the active culture supernatants. (3) Treatment of ticks with the pure compound, oxalate, at pH 4.0 resulted in almost 80% mortality in adult A. americanum ticks within 14 d, whereas treatment of ticks with oxalate at pH 7.0, or with formate, citrate, or phosphate at pH values of both 4 and 7 resulted in less than 10% mortality even after 28 d. (4) Cell-free culture supernatants from B. bassiana mutants with decreased oxalate production displayed lower acaracide activity than wild-type.

Iron acquisition in the entomopathogenic fungus Beauveria bassiana

Greg Westwood and Nemat O. Keyhani

University of Florida, Microbiology and Cell Science, Gainesville, FL 32611, USA

The ability to acquire iron in vivo is essential for most microbial pathogens. Iron assimilation by fungi can involve siderophore production and subsequent siderophore-iron transport as well as reductive iron assimilation systems. Beauveria bassiana is an entomopathogenic fungus currently under extensive study as a biocontrol agent of a broad range of arthropods. Despite this interest, little is known concerning the molecular mechanisms of B. bassiana mediated pathogenicity, especially in regards to iron acquisition. HPLC and mass spectroscopy analysis of purified iron-binding...
fractions derived from B. bassiana revealed a conidial storage molecule with a molecular mass of 770 that was identified as ferrococin. Analysis of the secreted fraction revealed several compounds ranging in molecular mass from 600-3200. A full-length cDNA clone corresponding to a putative B. bassiana siderophore transporter was identified and cloned into a yeast expression vector. The B. bassiana clone was used to functionally complement a yeast siderophore transport mutant. These results demonstrate that B. bassiana contains a suite of siderophores as well as specific systems for iron uptake.

**Competition of different methods for isolation of entomopathogenic fungi from soil**

Wondirad Mandelto1, 2Mohammed Dawd1 and Svetlana Gouli2

1Ethiopian Agricultural Research Organization, Ambo, Ethiopia
2Entomology Research Laboratory, University of Vermont, Burlington, USA

Five different methods for isolation of the entomopathogenic fungi from soil including the bait (BM), soil dilution (SDM), direct inoculation (DIM), print (PM) and electrostatic methods (ESM) were compared. Old-arable soil and forest soil were used for these experiments. Both soil types were artificial contaminated with two different fungi, - Beauveria bassiana and Metarhizium anisopliae (each fungus - 1000 conidia per 1 g soil). Wax moth - Galleria mellonella larvae were used for the BM. For SDM initial soil suspensions (10g soil per 200 ml water) and two tenfold dilutions - 1:10 and 1:100 was applied. In case of the DIM 10 mg soil was mixed with 0.15 ml sterile water and soil suspension was distributed evenly on surface of special selective media for B. bassiana and M. anisopliae. The PM is done with the mechanical transfer of small particles using wet flat bottomed cylinder (4 cm) from soil surface to cultivation medium. For ESM the microscopic soil particles were transferred using the electro statically-charged plastic materials. Isolation of the fungi was done on two semi-selective nutrition media transferred using the electrostatically-charged plastic materials. Colonies obtained was following: SDM - 50.6% for B. bassiana and M. anisopliae. For the arable soil number of colonies obtained was following: SDM - 50.6% for B. bassiana, 55.5% for M. anisopliae; DIM - 31.6% and 22.2%; PM - 12.6% and 5%; ESM - 5% and 0% respectively. Whereas from the forest soil: SDM - 31.6% and 22.2%; DIM - 5.1% and 0%; PM - 7.6% and 0%; ESM - 5% and 0% respectively were obtained. The fungi did not recovered using the bait method in both soils except numerous non-target fungi. Predominant fungal species from the arable soil was Fusarium sp., in case of the forest soil it was Paecilomyces lilacinus and Trichoderma sp. The soil dilution method allows isolate the different species of fungi in different type of soil. It also allows estimate the contamination level of soil.

**Virulence and sporulation of Metarhizium anisopliae in the presence of Trichoderma conidia on agar substrates and in soil**

Linda Hjeljord1 and Richard Meadow2

1Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Aas, Norway, The Norwegian Crop Research Institute, Høgskolevegn. 7, N-1432 Aas, Norway

Interactions between Metarhizium anisopliae and the soil microflora may affect the persistence of the insect pathogen. Among the most common soil fungi are Trichoderma spp. Some of these species may be applied to plants or soil for biological control of plant diseases. We have previously reported that Metarhizium conidia were unable to establish colonies on nutrient substrates if inoculated with T. atroviride at 20-22°C. In the present investigation we wished to determine whether Metarhizium conidia were inhibited at other temperatures, and whether virulence of M. anisopliae was impaired on larvae in the presence of Trichoderma. In vitro tests on agar showed that M. anisopliae was most resistant to overgrowth by Trichoderma at temperatures below 20°C, despite germination and growth optimus at 25-30°C. When black vine weevil larvae were dipped in conidial suspensions of M. anisopliae alone or mixed with conidia of Trichoderma and incubated in soil at 22°C, the ability of the insect pathogen to infect and kill the larvae was not affected by the presence of Trichoderma conidia in the inoculum. Trichoderma colonies were found only in soil or on larval sheddings. M. anisopliae in suspension together with Trichoderma conidia gave similar mortality to M. anisopliae alone.

**Spodoptera littoralis response to infection with AcMNPV**

Hadasash Rivkin1, Jeremy A. Kroemer2, Bruce A. Webb2 and Nor Chejnovsky3

1Entomology Department, Institute of Plant Protection, The Volcani Center, POB 6 Bet Dagan, 50250, Israel, and 2Department of Entomology, University of Kentucky, Lexington, KY 40546-0091, USA

We studied the infection of S. littoralis larvae by the Autographa california nucleopolyhedrovirus (AcMNPV) utilizing vAchspGFP, a polyhedra - positive recombinant that expressed GFP gene under the control of the hsp70 promoter from Drosophila. Oral infection of 4th instar S. littoralis larvae resulted in no mortality. At 24 h postinfection the midgut columnar cells of 40-60 % of the larvae showed viral foci with a high degree of melanization colocalized with GFP fluorescence. At 72-96h postinfection all foci were cleared and no expression of GFP could be detected. Injection vAchspGFP budded virus to the prolong S. littoralis larvae showed viral GFP-foci without melanization in fat body tissue and tracheal branches joints that propagated through these tissues at 72 h post-infection and resulted in larval death. A low extent of GFP-signaling (4 to 13%) was observed in hemocytes. 4th instar S. littoralis larva with immunosuppressed by the endoparasitic wasp Chelonus inanitus became more susceptible to AcNPV infection. Moreover, bioassays showed that the infectivity towards S. littoralis larvae of a recombinant AcMNPV expressing an immunosuppressive polydnavirus gene was 10-fold higher than that of wild type AcMNPV. These data indicate that S. littoralis mounts an immune response against AcMNPV and that immunosuppression can enhance AcMNPV’s pathogenicity towards S. littoralis.

**Hemocyte variations relating to age related immunocompetancy in gypsy moth (Lymantria dispar)**

Jim McNeil, Diana Cox-Foster, Mike Grove, Kelli Hoover

Department of Entomology, The Pennsylvania State University, University Park, PA 16802, USA

One of several barriers to the effective use of the baculovirus Lymantria dispar nucleopolyhedrovirus (LdNPV) to control gypsy moth (Lymantria dispar) is susceptibility to viral infection varies within an instar. A viral LD80 for newly molted larvae will only kill about 40% of larvae infected at 48-72 hours post-molt, a phenomenon called intrastadial developmental resistance. Our hypothesis is that this pattern is driven by differences in the ability to mount anti-viral defenses. To begin exploring the basis of this resistance, we examined differences in hemocyte numbers, composition, and behavior between susceptible and resistant aged gypsy moth larvae. We assessed total hemocyte numbers and composition from hemolymph samples collected at different times post-inoculation. Hemocyte behavior was observed by making time-lapse movies of hemocytes. Although there were no significant differences in total hemocyte numbers between susceptible and resistant aged larvae, there were differences in the proportions of one hemocyte type, the oenocytoids, which are thought to be involved in the phenoloxidase cascade. There were significantly more oenocytoids in resistant aged larvae. Additionally, resistant aged larval hemocytes were more immunologically active than hemocytes from susceptible-aged larvae. These results may provide leads to the basis for intrastadial developmental resistance in gypsy moth to LdNPV.
Molecular cloning of *Choristoneura fumiferana* prophenoloxidases 1 and 2 and their regulation by a polydnavirus

**Daniel Douce**1, Qili Feng2 and Michel Casson1

1Laurentian Forestry Centre, Natural Resources Canada, 1055 rue du PEPS, Quebec, Canada, 2Great Lakes Forestry Centre, Natural Resources Canada, 1219 Queen Street East, Sault Ste. Marie, Canada

Melanization plays a significant role in the immune response of insects against parasites as it supplements the cellular encapsulation by hemocytes. The enzyme pro-phenoloxidase (PPO) is responsible for the formation of melanin. Suppression of host PPO activity is a strategy deployed by numerous insect parasites, yet the mechanisms employed and the significance of this immune suppression are not well understood. A previous study by our team determined that the parasitic wasp *Tranosema rostrale*, which lays its eggs in larvae of *Choristoneura fumiferana*, suppresses hemolymph melanization and PPO activity. This inhibition of PPO activity in *C. fumiferana* can be reproduced by injecting in the hemocoele a polydnavirus produced by the wasp (the *T. rostrale* ichnovirus, TrIV). To gain insight into the molecular mechanisms of PPO inhibition by TrIV, we cloned and sequenced the two PPO genes of *C. fumiferana* (CfPPO). Partial CfPPO sequences were retrieved from a *C. fumiferana* expression sequence tag (EST) database. In order to clone the complete cDNAs for CfPPO and CfPPO2, 5′- and 3′- RACE experiments on a *C. fumiferana* hemocyte cDNA library were attempted. Results from the cloning experiments as well as the transcriptional regulation of CfPPOs by TrIV will be presented.

Contributed paper. Wednesday, 8:45.

Polydnavirus-induced apoptosis of host hemocytes after parasitization of the host lepidopteran *Manduca sexta* by the parasitoid wasp *Cotesia congregata*

Ronald F. Dumps1 and Nancy E. Beckage2

1Department of Biochemistry and Molecular Biology, University of California-Riverside, Riverside, CA 92521, 2Departments of Entomology & Cell Biology and Neuroscience, University of California-Riverside, Riverside, CA 92521, USA

*Cotesia congregata* is an endoparasitic wasp that develops in the tobacco hornworm, *Manduca sexta*. Parasitization occurs when the female *Cotesia* wasp injects her eggs mixed with venom and polydnavirus (PDV) into the host caterpillar. PDV-encoded proteins are expressed beginning immediately after parasitization, which triggers apoptosis (programmed cell death) of host hemocytes and host immunosuppression. Expression of the PDV protein, CrV1, which was first isolated in *Cotesia rubecula*, appears to accompany host immune suppression necessary for parasitoid survival. Morphological changes such as rounding up and nuclear compaction in hemocytes from a parasitized *M. sexta* larva were observed using fluorescence microscopy, to follow the temporal progression of events leading to apoptosis. Antibody staining correlated the presence of CrV1 homolog protein (CcV1) in these apoptotic hemocytes. We further compared the number of apoptotic hemocytes from a parasitized *M. sexta* using a TUNEL assay with healthy *M. sexta* hemocytes. Hemocyte viability was determined through BrdU-labeling to investigate differences between parasitized and non-parasitized hemocytes. We have identified a viral-encoded protein from the *C. congregata* polydnavirus (CcV1) that participates in mediating cell death of parasitized hemocytes. We have identified a viral-encoded protein from the *C. congregata* polydnavirus (CcV1) that participates in mediating cell death of parasitized hemocytes. Partial CfPPO sequences were retrieved from a *C. fumiferana* expression sequence tag (EST) database. In order to clone the complete cDNAs for CfPPO and CfPPO2, 5′- and 3′- RACE experiments on a *C. fumiferana* hemocyte cDNA library were attempted. Results from the cloning experiments as well as the transcriptional regulation of CfPPOs by TrIV will be presented.

Contributed paper. Wednesday, 8:30.

Genomic analysis of the *Drosophila melanogaster* innate immune response against a parasitic wasp

Shannon Albright and Dan Hultmark

Umea Centre for Molecular Pathogenesis, Umea University, 90187, Umea, Sweden

The innate immune system of *Drosophila* has both humoral and cellular aspects. Many of the key players involved in the humoral response have been identified in genetic screens and microarray analyses (reviewed in Govind and Nehm, 2004). However, far less is known at the molecular level about the cellular immune response. The cellular response involves phagocytosis or encapsulation of pathogens by hemocytes (blood cells). The latter response can be triggered by parasitic wasp infestation. In order to get a better understanding of the transcriptome underlying an antiparasitic immune response, a timecourse microarray analysis has been performed following infestation of larvae by *Leptopilina bouardi*, a natural parasite of *Drosophila*. The infection-dependent genes that have been identified contribute to several biological processes including immune and stress responses. Similar to the bacterial immune response, a large proportion of the transcripts identified are serine proteases. Interestingly, the majority of these proteases have not been previously associated with an immune response, indicating that the induction of the response may be fundamentally different from other immune challenges.

Contributed paper. Wednesday, 9:15.

Investigating immune functions in mosquito cell lines

Ann M Fallon

Department of Entomology, University of Minnesota, St. Paul, MN 55108, USA

Mosquito cell lines provide a tool for identifying immune-induced proteins and peptides. The well-characterized C7-10 cell line from *Aedes albopictus* is highly phagocytic, and secretes transferrin, cecropin, defensin and lysozyme, as well as additional unidentified proteins, in response to microbial challenge. To gain insight into how phagocytic activity interfaces with induction of immune proteins, we seek to identify conditions under which phagocytosis is inhibited. Phagocytosis occurs in the presence of the DNA synthesis inhibitor, hydroxyurea, the RNA-synthesis inhibitor, actinomycin D, and the protein synthesis inhibitor, cycloheximide, suggesting that the phagocytic machinery is not dependent on DNA, RNA or protein synthesis. Preliminary data indicate that phagocytosis is partially inhibited by cytochalasin, a drug which disrupts the cytoskeleton. Phagocytosis is most effectively inhibited by heat shock. Future work will explore optimal conditions of temperature and time for inhibition of phagocytosis and determine the reversibility of the response.

Contributed paper. Wednesday, 9:30.

Transcriptome studies on the penaeid shrimp biodefense-related genes

Takashi Aoki1, Ikuo Hirono1, Motoshige Yasuike1, Koolvara Sangrungrung2, Ryuji Ueno1, Lila Ruangpan2, Yukinori Takahashi2, Ratree Wongpanya3, and Anchaalee Tassanakajon4

1Tokyo University of Marine Science and Technology, Japan, 2Kung Krabae Bay Fisheries Development Study Centre, Thailand, 3Mie University, Japan, 4Chanthaburi Coastal Fisheries Research and Development Center, Thailand, 5National Fisheries University, Japan, 6Chulalongkorn University, Thailand

The shrimp possesses an innate immunity that is composed of both humoral and cellular responses. However, little is known about this system particularly the mechanisms involved at the molecular level. Recently, we conducted an expressed sequence tags (ESTs) analysis of the kuruma shrimp, *Penaeus monodon* to discover immune-related genes. Based on these ESTs, we constructed a cDNA microarray (spotted 1,026 ESTs) and performed following infestation of larvae by *Vibrio harveyi* or *Vibrio harveyi* by using microarray technique. The number of genes in the black tiger shrimp hemocytes showed changes in their expression levels after administration of antibiotics. In the case of *Vibrio harveyi*, several gene expressions were changed, i. e.
expression patterns in the shrimp hemocytes were different between bacterial and viral infection.

Contributed paper. Wednesday, 9:45. 110 Characterization and expression analysis of biodefense-related genes from kuruma shrimp, Marsupenaeus japonicus
Ikko Hirozo and Takashi Aoki
Laboratory of Genome Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo 108-8477, Japan
Basic knowledge of shrimp immunity is needed to develop strategies for prophylaxis and control of diseases in shrimp aquaculture. To create a better understanding of shrimp immunity, this study was undertaken to clone and characterized α2-macroglobulin (α2M) and crustin-like peptide (antibacterial peptide) from kuruma shrimp Marsupenaeus japonicus. The cDNA encoding the M. japonicus α2M contains an open reading frame of 4,518 nucleotides that translate into a 1,505 amino acid putative peptide. In a healthy shrimp, the mRNA of α2M was mainly expressed in the haemocytes. Five variants (types 1 to 5) of M. japonicus crustin-like peptide cDNAs were obtained from a haemocyte cDNA library. M. japonicus crustin-like peptide type 1 has a cDNA of 679 nucleotides and an open reading frame (ORF) of 573 bp coding for 191 amino acid residues. Other types contained varying glycine-rich repeats at the N-terminal amino acid sequences. Expression of M. japonicus crustin-like peptide mRNA was detected in haemocytes, but not in heart, hepatopancreas, gill, fore-gut, mid-gut, muscle, subcuticular epithelium or ovary. The expression of α2M and crustin mRNA were dramatically increased after peptidoglycan (PG) administration.

SYMPOSIUM (Cross Divisional). Wednesday, 10:30-12:30
Invertebrate responses to pathogens
Symposium. Wednesday, 10:30. 111
B. thuringiensis, pore-forming toxins, and their interactions with C. elegans
Danielle L. Huffman, Larry J. Bischof, David LaHaie, Wayne Hsu and Raffi V. Arayan
Division of Biological Sciences, University of California San Diego, La Jolla, CA, 92039-0349, USA
Pore-forming toxins (PFTs) constitute one of the most important single classes of bacterial virulence factors. Bacillus thuringiensis (Bt) crystal (Cry) toxins are PFTs famous for their ability to target insects and nematodes. We are using the interaction of Bt and Bt Cry toxins with the genetically-tractable nematode C. elegans to study how animals respond to and defend against PFTs and pathogenic Bacilli. Using Affymetrix gene chip, we have shown that the C. elegans transcriptome responds rapidly and robustly to PFT. One of the pathways up-regulated is p38, a pathway important for innate immunity in mammals. Loss of p38 in C. elegans leads to animals that are hypersensitive to attack by Crystal toxin. The p38 pathway is also shown to play an important role in defending mammalian cell systems against PFTs. We identified 3 downstream targets of the p38 pathway, some of which are important for protecting C. elegans against PFTs made by mammalian pathogens. We are exploring whether and how the p38 pathway is activated in response to PFT. Independently, we are using forward and reverse genetics and are uncovering many new genes and pathways involved in defense against PFTs. Our goal is to understand how animals defend against intoxication by PFTs.

Symposium. Wednesday, 11:00. 112 Identification of a gene family in Spodoptera exigua expressed in the midgut in response to pathogens: Cross-talk between responses to Bt toxin and to baculovirus
Salvador Herrera1, 2, Marleen Ansems3, Monique M. van Oers4, Just M. Vlak3, Petra L. Bakker4, William J. Moar5, and Ruud A. de Maagd6
1Plant Research International B.V., Wageningen, the Netherlands, 2Laboratory of Virology, Wageningen University, the Netherlands, 3Laboratory of Genome Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo 108-8477, Japan, 4Laboratory of Virology, Wageningen University, the Netherlands, 5Laboratory of Genome Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo 108-8477, Japan, 6Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama 36849, USA
The response of insects to pathogens involves changes in gene expression, which may help the insect to overcome the effects of pathogens or their toxins. In our current work, Suppression Subtractive Hybridization (SSH) was used to make cDNA-fragment libraries of genes that are up- or down-regulated in the midgut of last instar larvae of the beet armyworm, Spodoptera exigua, when exposed to the Bacillus thuringiensis (Bt) Cry1Ca toxin. Subsequent microarray studies using these libraries revealed altered gene expression levels for several genes in response to Bt toxin exposure. Among the genes upregulated after feeding with Bt toxins a new family of genes (Repat genes from “response to pathogen”) was identified. cDNA’s from four members of the Repat family were cloned and sequenced. Additional studies revealed that this gene family was also up-regulated during infection with the baculovirus Autographa californica (Ac) MNPV. A recombinant AcMNPV virus expressing Repat1 was constructed and used to infect S. exigua larvae. The pathogenicity of this Repat1 recombinant baculovirus was reduced compared to the control confirming the role of Repat1 in reducing detrimental effects of pathogen infection in the larvae.

The infection and cell specific replication of the most successful viral insecticide, Anticarsia gemmatalis nucleopolyhedrovirus (AgMNPV), in its host Bergmann M. Ribeiro
Departamento de Biologia Celular, Universidade de Brasilia, Brasilia DF, CEP 70910-900, Brazil
The baculovirus Anticarsia gemmatalis Multiple nucleopolyhedrovirus (AgMNPV) is the world most successful viral pesticide and is being used in Brazil for the control of the soy bean pest, the velvetbean caterpillar, A. gemmatalis. We have used recombinant AgMNPV viruses to follow the AgMNPV infection and replication inside insect larvae and shown that AgMNPV is able to infect most tissues of its host. We have also characterized A. gemmatalis haemocytes and AgMNPV replication in these cells by light and electron microscopy. Furthermore, we have demonstrated that a mutant AgMNPV derived virus (vApAg) is capable of inducing apoptosis in vivo by accessing vApAg infection in A. gemmatalis haemocytes by intraheamocoeolic inoculation. vApAg also induces apoptosis in a cell culture derived from A. gemmatalis (UFL-Ag-286), arrogating protein synthesis at late times post-infection and reducing viral progeny production. Apoptotic bodies and entire cells were phagocytosed by phagocytic cells and granulocytes, and necrosis of infected cells was also observed. The average time of death was extended for vApAg-infected larvae if compared to the time of death by infection with the wild-type virus. These results show correlation of apoptosis occurrence in vivo and the reduced infectivity of vApAg in A. gemmatalis larvae.

Immune depression triggered in insects by the bacteria Xenorhabdus nematophila and Photorhabdus luminescens
Michel Brehelin, Robert Zumbihl, Karine Brugirard, Fabienne Vigneux, Carlos Ribeiro and Alain Grivaud
Ecologie Microbienne des Insectes et Interactions Hôtes-Pathogènes, INRA-UMI PI E. Bataillon 34095, Montpellier, France
Enterobacteriaceae of the genus Xenorhabdus and Photorhabdus are potent pathogens of a large spectrum of insect species (Boemare N., 2002, in “Entomopathogenic nematology”, Gaugler R ed., CABI pub., pp. 35-56) some strains of which are emerging pathogens for human (Gerrard J. et al., 2004, Microbes and Infection, 6, 229-237). Insect larvae die in a few days after infection. Because Xenorhabdus and Photorhabdus septicemia arises in the insect body, it is obvious that these bacteria are able to escape defense reactions and especially the cellular ones that are early settled after the pathogen penetration. Whereas inhibitors of the PO system (Brehelin M. et al., 1989, Insect Biochemistry, 19, 301-307) and of eicosanoids (Park Y. and Kim Y., 2000, Journal of Insect Physiology, 46, 1469-1476) are secreted by...
these entomopathogenic bacteria, the means by which they escape the defense reactions are very poorly understood. In this review we show that different toxins are secreted by these bacteria and have the insect immune systems (haemocytes) as main targets. There is a high redundancy in the kinds of secreted immunodepressive toxins and in their modes of action.

**NEMATODES**

Contributed paper. Wednesday, 10:30. 115

*Do entomopathogenic nematodes have potential as biological control agents of stored product insects?*

James E. Campbell1, Olga Ramos-Rodriguez2, and Sonny Ramaswamy2

1USDA ARS GMPRC, 1515 College Ave, Manhattan, KS 66502, USA, 2Department of Entomology, Kansas State University, Manhattan, KS 66506, USA

Entomopathogenic nematodes at first appear poorly adapted for use as biological control agents against insects that infest grain and processed grain-based products. However, the population structure and movement patterns of stored product insects indicate that in many environments pests originating from food material that accumulates in hidden refugia -- such as cracks or crevices in structures, empty grain bins, spillage outside facilities -- and subsequent movement into stored commodities can be important factors in product infestation. Biological control in general, and entomopathogenic nematodes in particular, may be an effective component of an IPM program when targeted at these refugia populations. We evaluated the efficacy of three Steinernema spp. against a range of stored product pest species and stages under laboratory conditions and found many pest species were susceptible. Tests of *S. riobrave* pathogenicity as a biopesticide under simulated field conditions, suggested that nematodes applied in a manner similar to some conventional chemical pesticides had a sufficient window of time to find and infect insects. Further research to enhance efficacy, evaluate range of field situations where nematodes might be used, and determine the impact of refugia treatments on overall pest population levels is still needed.

Contributed paper. Wednesday, 10:45. 116

*Control of navel orangeworm in fallen pistachios using large scale application of the entomopathogenic nematode, Steinernema carpocapsae*

Joel P. Siegel1, Lawrence A. Lacey1, Patricia Noble1, James Bettiga3, Bradley Highes2, and Robert Fritts, Jr.1

1USDA/ARS, SJVASC, Parlier, CA 93648, 2USDA/ARS, YARL, Wapato, WA 98951, 3US & J Ranch, Madera, CA 93638, 4Paramount Farming Company, Bakersfield, CA 93308, 5Certis USA, Columbia, MD 21046, USA

Previously, we demonstrated that in small plot studies, infective juveniles (IJ$s$) applied at a density of one billion per hectare and application rates of 1,800-3,740 liters per hectare followed by wetting rates. In all three nematodes

Penetration rates in single-piece sand columns significantly differed in the three modified strains was more than 10 times greater.

Valko showed intermediate penetration rates but not adversely affect each other at all depths. S. carpocapsae NY001 was not affected negatively at 0 cm by either of the other nematodes and was not affected by *H. bacteriophora* Oswego at 32.5 cm. The performance of *S. feltiae* Valko was positively affected at 26 cm and 32.5 cm depths and the performance of *H. bacteriophora* Oswego was positively affected at 32.5 cm by the presence of *S. carpocapsae* NY001. *S. feltiae* Valko was not affected by *H. bacteriophora* Oswego at 0 cm and 32.5 cm depths but *S. feltiae* Valko had more negative effect on *H. bacteriophora* Oswego at and below 19.5 cm than *S. carpocapsae* NY001.
that bacterial transfer and hybridization could be a valuable tool in improving biocontrol efficacy of steer nematodids.

**STU** Contributed paper. Wednesday, 11:30. 119

*Evaluating efficacy of application of entomopathogenic nematodes in drip-line irrigation systems*

Andrew P. Brown1, Jeremy D. Pearce2 and Denis J. Wright1

1Division of Biology, Faculty of Life Sciences, Imperial College London, Silwood Park campus, Ascot, Berkshire, SL3 7PY, UK, 2BeckerUnderwood, Harwood Industrial Estate, Littlehampton, West Sussex BN17 7AU, UK

A major constraint for entomopathogenic nematodes (EPN) in biocontrol is uneven distribution during application. Improvements in application to give more even emission along drip irrigation lines will lead to more efficient control and improve the marketability of EPN. A key factor influencing distribution during application is nematode settling, especially in slow release methods, such as drip-line irrigation. Using a 100 m test irrigation rig we have shown that although some irrigation tapes give a constant release of water, EPN emission decreases with increased distance along the tape. The distal end of the irrigation line was identified as a dead zone with little or no EPN released. Settling of EPN was the principal cause of decreased output. The effects of different tape types and specifications (flow rate and diameter), EPN species, tank mix additives and mechanical agitation on the rate of nematode settling was investigated. We showed that the length of the dead zone was not directly proportional to tape length and that the key factor was the internal flow rate. Modelling has been used to explain these observations and to help interpret observations in commercial systems. This work should lead to improved protocols for EPN application in drip-line irrigation systems.

**STU** Contributed paper. Wednesday, 12:00. 121

*Infection preferences of an entomopathogenic nematode, Steinernema riobrave*

Jayne M. Christen1, James F. Campbell2, and Sonny B. Ramaswamy1

1Kansas State University, Department of Entomology, Manhattan, KS 66506, USDA-ARS, Grain Marketing and Production Research Center, 1515 College Ave, Manhattan, KS 66502, USA

Entomopathogenic nematodes are lethal endoparasites of insects. Infective juveniles (IJ) actively forage and infect new hosts. Although much is known about how IJs search for hosts, less is known about the infection process. Infective juveniles may encounter unparasitized insects or insects already parasitized by conspecific or heterospecific entomopathogenic nematodes. Quality of an insect as a resource will change depending on type and stage of parasitization. The influence of parasitization status on host acceptance and infection by IJs was determined using no choice and choice experiments. Steinernema riobrave and two host insect species, Galleria mellonella and Tenebrio molitor, were used in experiments. Results from no choice experiments indicate that host infection declines after initial parasitization. However, 72 h after initial parasitization IJs continued to infect parasitized hosts. In choice assays, S. riobrave showed preference for 24 h infected larvae over uninfected and 48 h infected larvae for G. mellonella and T. molitor, respectively. Preference for recently parasitized hosts may be adaptive under certain conditions because of increased probability of establishing and reproducing, but could negatively impact efficacy as biological control agents when used as a biopesticide.
**MICROBIAL CONTROL 1**

**Contributed paper. Wednesday, 10:30. 123**

**Sphaerus® SC, a Brazilian bioinsecticide to control the vector of malaria and filariases**

Rose Monnerat and Carlos Marcelo Soares

1Embrapa Recursos Genéticos e Biotecnologia, CP 02372, Brasília, DF, Brazil, 2BtBek Biotecnologia Ltda. SAAN. QDR. 3 lote 240, Brasília, DF, Brazil

Sphaerus® SC is a bioinsecticide developed through a cooperation between Embrapa and BtBek Biotechnology, a Brazilian enterprise. This product is based on a *Bacillus sphaericus* strain isolated from Brazilian soil, named S242 from Embrapa’s Culture Collection of *Bacillus* spp. strains. This strain is fermented in a medium made of agro industrial residues and formulated as a concentrated suspension. The product has 2.5% of active ingredient, 60 ITU and is registered at Brazil’s Health Ministry under the number 3.2813.0001.001-5. It has been used to control *Anopheles* spp. larvae in the Amazon region persisting for 2 weeks in fish nurseries and in the southern region of Brazil in sewerage ponds and rivers controlling 100% of *Culex quinquefasciatus* larvae for 30 days.

Contributed paper. Wednesday, 10:45. 124

**Bt-horus® SC, a Brazilian bioinsecticide to control mosquitos and black-flies**

Rose Monnerat and Carlos Marcelo Soares

1Embrapa Recursos Genéticos e Biotecnologia, CP 02372, Brasília, DF, Brazil, 2BtBek Biotecnologia Ltda. SAAN. QDR. 3 lote 240, Brasília, DF, Brazil

Bt-horus® SC is a bioinsecticide developed through a cooperation between Embrapa and BtBek Biotechnology, a Brazilian enterprise. The product is based on a *Bacillus thuringiensis israelensis* strain isolated from Brazilian soil, named S1806 from Embrapa’s Culture Collection of *Bacillus* spp. strains. This strain is fermented in a medium made of agro industrial residues and formulated as a concentrated suspension. The product has 1.2% of active ingredient, 1,200 ITU and is registered at Brazil’s Culture Collection of *Bacillus* spp. strains. It was strain is fermented in a medium made of agro industrial residues and formulated as a concentrated suspension. This strain is strain that has 1.2% of active ingredient, 1,200 ITU and is registered at Brazil’s Health Ministry under the number 3.2813.0001.001-5. It has been used to control *Anopheles* spp. larvae in the Amazon region persisting for 2 weeks in fish nurseries and in the southern region of Brazil in sewerage ponds and rivers controlling 100% of *Culex quinquefasciatus* larvae for 30 days.

Contributed paper. Wednesday, 11:00. 125

**Controlled delivery of single and joint-action biodilavicide formulations for control of mosquito larvae**

Richard Levy, Michael A. Nichols, and William R. Opp

Lee County Mosquito Control District, Technology Development Center, P.O. Box 60005, Ft. Myers, FL 33906, USA

Bioassays were conducted with single and joint-action Matricap® controlled delivery formulations of *Bacillus thuringiensis var. israelensis (B.t.i.)* and *B. sphaericus* that were coated/impregnated on granular matrices for sustained control of mosquito larvae in fresh and brackish water. Joint-action mixtures of *B.t.i.* and the insect growth regulators pyriproxyfen or methoprene were also evaluated. Product mixtures were utilized to evaluate possible efficacy enhancement. Also, mixing products with different toxins and/or modes of action can be an excellent tool for resistance management. Corb cob granules were used as carriers and fatty acid, fatty alcohol, fatty acid ester, fatty alcohol ester, phthalyl ester, wax, or plasticizer coatings were used as controlled release regulators. The release kinetics of bioactive agents from a granular matrix were functions of hydrolysis, solubility, melting point, biodegradation, photodegradation, and specific gravity of the coating/coating complex that was admixed with a carrier and bioactive agent. By selecting coatings with specific physicochemical characteristics that complement or modify the physicochemical characteristics of the carrier and/or bioactive agent, a controlled delivery system can be designed to target the feeding and orientation patterns of mosquito larvae in specific surface and/or subsurface areas of an aquatic habitat.

Contributed paper. Wednesday, 11:15. 126

**Decreased resistance to Bt cotton in pink bollworm?**

Aaron J. Gassmann, Jeffrey A. Fabrick, Mark S. Sisterson, Shai Morin, Timothy J. Dennehy, Yves Carrière, and Bruce E. Tabashnik

Department of Entomology, University of Arizona, Tucson, AZ 85721, USA

Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins reduce reliance on insecticides, but evolution of resistance by pests could cut short their usefulness. Pink bollworm is a major pest that has experienced intense selection for resistance to Bt cotton in Arizona since 1997. Unexpectedly, monitoring data from bioassays show that the frequency of pink bollworm resistance to Cry1Ac, the toxin in Bt cotton, decreased from 1997 to 2003. Field-based estimates also show sustained efficacy during this period. In laboratory-selected strains that can survive on Bt cotton and have up to 3,100-fold resistance to Cry1Ac, resistance is linked with three recessive mutations in the gene encoding a cadherin protein that binds Cry1Ac. DNA-based monitoring of field-sampled individuals using the polymerase chain reaction (PCR) indicates that the frequency of the three identified resistance alleles remains low. A new synthesis of experimental and modeling factors suggests that key factors causing the decline in pink bollworm resistance to Bt cotton are refuges of cotton without Bt toxin, recessive inheritance of resistance, incomplete resistance, and fitness costs associated with resistance.

Contributed paper. Wednesday, 11:30. 127

**Evaluation of two formulations based on microbial metabolites to the control of blackcurrant insect pests**

Margarita V. Shternshe1, Maxim V. Vaskin1, Vladimir V. Govii2

1Novosibirsk State Agrarian University, Russia, and 2University of Vermont, USA

Two formulations registered in Russia were tested to the control of Siberian populations of blackcurrant insect pests: gooseberry fruitworm, *Zophodia convolutella*, gooseberry aphid, *Aphis glossularia* and currant aphid, *Capirophoros ribis*, under laboratory and field conditions. Lepidocide® based on spores and endo-toxin crystals of *Bacillus thuringiensis subsp. kurstaki*, and Phytoverm® based on avermectin complex isolated from *Steptomyces avermitilis* biomass were used for evaluation. Laboratory experiments showed that a treatment of *Z. convolutella* larvae with both formulations at concentration 0.2-0.4 % led to the significant larval mortality up to 94% after 72-96 hours. Phytoverm® had shortened the period of mortality in comparison with Lepidocide®. In addition, Phytoverm® had caused a high mortality of both species of aphids after 12-48 hours. Field testing of two formulations was carried out during 2003-2004. Blackcurrant bushes infested by insect pests were sprayed with Lepidocide® or Phytoverm®. Two treatments were needed for achievement of the significant efficacy of both formulations for *Z. convolutella*, and Phytoverm® for *A. glossularia*. These treatments had provided suitable protection of plants, and the efficacy of both formulations was comparable with synthetic insecticide which traditionally applied on the black currant. In addition, the most common beneficial insects in blackcurrant community - predatory ground beetles (Coleoptera, Carabidae) - served as an indicator species of ecological safety. The treatment with synthetic chemical insecticides is strongly suppressed the number of ground beetles for a long period. The Phytoverm® treatment led to some suppression with the tested conditions.
Mortality of gypsy moth (Lymantria dispar) induced by Bacillus thuringiensis var. kurstaki is inversely related to temperature

Kees van Frankenhuysen

Great Lakes Forestry Centre, Canadian Forest Service, 1219 Queen Street East, Sault Ste. Marie, Ontario, P6A 2E5 Canada

Despite 3 decades of operational use of Bacillus thuringiensis for management of gypsy moth populations, little is known about the processes underlying its efficacy. Larval responses to sublethal and lethal doses of Foray 48B were investigated as a function of temperature and instar. Sublethally dosed larvae ceased feeding for a period that depended on dose, temperature and instar. Feeding inhibition of 10-15 h was observed at dose levels as low as one tenth of the LD_{50}. Time to recovery of third instars dosed with a LD_{50} increased from ~20 h at 25°C to ~80 h at 13°C. The 50% lethal dose (LD_{50}) ranged from 0.02 International Units (IU) for first instars to 2.0 IU for fourth instars. Larval mortality progressed rapidly and was complete within 3 (first instars) to 4 (fourth instar) days after dosing at 22°C. Rearing temperature was varied from 13 to 25°C and had a profound effect on mortality. In each larval stage, mortality progressed more rapidly at higher temperatures, but the maximum level of mortality attained was inversely related to temperature. Mortality always occurred during logarithmic growth of vegetative cells, well before onset of the stationary phase. The possible role of vegetative insecticidal proteins in causing the observed mortality patterns will be discussed.

**STU** Contributed paper. Wednesday, 12:00. 129

Cloning and expression of cry1Ah1 gene from isolate of Bacillus thuringiensis and its bioactivity

Haitao Li¹,², Hanxin Tan¹, Lanlan Han², Kangli He¹, Gencai Liang¹, Fuping Song¹, Dafang Huang¹, Jie Zhang¹

¹State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, ²College of Life Sciences, Northeast Agricultural University, Harbin, 150030, Biotechnology Research Institute, CAAS, Beijing 100081, P. R. China

A novel Bacillus thuringiensis cry gene was cloned from wild isolate B-8-G screened from northeastern China. Its full length was 3549bp, deduced amino acids were 1182 with 1343kDa molecular weight. As a new holo-type cry gene, this gene was named cry1Ah1 (Accession number AF281866) by Bt 6-Endotoxin Nomenclature Committee. It could be expressed normally in Bt acrystalliferous mutant HD-73-6-5 and E. coli BL21 strain by different vectors respectively. Bioassay results showed that Cry1Ah1 toxin was very strong activity against lepidopterous larvae, cotton boll worm, corn borer, rice stem borer and diamond back moth. Lethal concentration 50% of Cry1A1h was much lower than Cry1Ac, Cry1Ab and Cry1Aa toxin against mentioned larvae respectively. This study result will benefit construction of genetically engineered bacterium and transgenic plant for biocontrol of significant insect pests of crop plants.

**STU** Contributed paper. Wednesday, 12:15. 130

Characterization of a Bacillus thuringiensis strain Bt185 toxic to the Asian cockchafer: Holotrichia parallela

Hong Yu¹,², Fuping Song¹, Jie Zhang¹, and Jiguo Gao²

¹State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, ²College of Life Sciences, Northeast Agricultural University, Harbin, 150030, P. R. China

A new Bacillus thuringiensis strain was isolated from HeBei soil samples and named Bt185 in China. Transmission electron microscopy observation demonstrated that the strain produced spherical parasporal inclusions similar to that of B. thuringiensis japonensis Buibui strain which showed toxicity to Anomala corpulenta and Popillia japonica. Plasmid profile on agarose gel revealed Bt185 contained six large plasmid bands of around 191kb, 1.61kb, 104kb, 84kb, 56kb and 37kb. SDS-PAGE analysis indicated Bt185 produced one major band with estimated molecular mass of 130-kDa. PCR-RFLP identification results showed that a novel cry8-type gene was found in Bt185 strain (unpublished data). When we screened the novel cry8-type gene, another novel cry8-type gene was isolated accidentally, and its partial sequence of 2340 bps was obtained and encoded a protein with 780 amino acids. Bioassays showed Bt185 had no toxicity against several Coleoptera and Lepidoptera pests. However, it exhibited toxicity against larvae of the Asian cockchafer Holotrichia parallela. This was the first report of the occurrence of a strain which had insecticidal activity to Holotrichia parallela larvae.

**Student Workshop and Mixer**. Wednesday, 12:30-2:00

Talking the talk: A “how to” guide

Workshop paper. Wednesday, 12:30. 131

The gestalt of performing: An eclectic guide to successful oral presentations

John D. Vandenberg

USDA ARS, U. S. Plant, Soil & Nutrition Lab., Tower Road, Ithaca, NY 14853, USA

How many times have you wanted to shout “Speak louder, please!”? Or has that been shouted and what came next were a few words you could understand followed by a quick return to mumbling? How often are you distracted by an untied shoelace? Or a stain on a shirt? Gone dizzy trying to follow a peripatetic pointer? Gotten drowsy listening to repeated “uh’s” and “um’s”? Felt like a speaker was talking to the screen, preaching at his/her data? There are countless aspects of making a successful presentation. I will try to identify a few of the key elements and give advice on how to overcome common problems. Presentations are performances. You assume a certain character and present rehearsed information to a willing audience. Though they may not have had to show their tickets at the door, your audience members certainly paid to be there. So give them their money’s worth! Preparation for your performance should include a certain amount of script writing, several rehearsals, costume design and make-up (or not!). During your show you will have to deal with lighting and sound. Your performance will need to be clearly seen and heard (and understood!). From pointer management to microphone mania. From elocution to elucidation. By conceiving of your oral presentation in performance terms, you can improve its impact and effectively communicate your exciting results.

Workshop paper. Wednesday, 12:42. 132

A good scientific researcher needs to be a good communicator

Alejandra Bravo

Instituto de Biotechnologia/UNAM, Cuernavaca, Mexico

One of the most important aspects of being a scientific researcher is to communicate your ideas and conclusions in a clear form, either written or orally. Therefore, training in these aspects is very important. In this presentation I will give my experience about how I learned to give scientific presentations of my data. The basic point in planning a presentation is to know what you want to communicate, and to know which type of audience will be listening. Then I can list a few key points in performing a presentation: 1.- Organize a complete history that best describes the data and ideas to be presented. 2.- Prepare clear figures without too much text. All figures have a purpose then include only those that are essential for your dissertation. 3.- End with a clear conclusion that the audience could acquire and remember for a long time.

Workshop paper. Wednesday, 12:54. 133

The WYSIWYG challenge: The visual aspects of presentations really DO matter

Richard A. Humber

USDA-ARS Plant Protection Research, US Plant, Soil & Nutrition Laboratory, Tower Road, Ithaca, NY 14853-2901, USA

The effectiveness of any presentation in a scientific meeting, classroom, or any other setting is dramatically affected by more than
just the content. The visual aspect of a poster or a slide-illustrated oral presentation can strongly reinforce or powerfully detract from the impact of the content or it can so distract the audience that the message of the content may be lost. WYSIWYG (“what you see is what you get”) computer interfaces and the sorts of choices they require of us pose powerful challenges for effective informational presentations since computers allow virtually infinite control over every visual nuance of a presentation. Taking control over such choices as the fonts, colors, backgrounds, and layouts of our visual aids and printed communications can vastly improve their impacts and effectiveness. As Marshall McLuhan said, "The medium is the message." No matter how much the meaning of that phrase is discussed, its practical impacts are seen everywhere around us: It is impossible to separate the means of presenting a message from its content. We can learn much and instruct others better by better “seeing” and understanding the impacts of our visual and design choices.

Workshop paper. Wednesday, 1:06. 134
Where art and precision meet: Presenting data clearly
Vince D’Amico
USDA Forest Service, Dept. Entomology & Wildlife Ecology, University of Delaware, Newark, DE 19716, USA

Face it, we all see a lot of incomprehensible tables and graphs projected onto the screen at SIP meetings. Not yours? Oh, OK. Just keep telling yourself that. That slide with 18 treatments has to be done just right if you want anyone to take away anything from it. And although a screen full of As, Ts, Cs, andGs may mean something to a blob ofrDNA sitting in the third row, everyone else is thinking, “wasn’t there a better way?” If Ed Tufte were available for our meeting, we could all sit back, relax, and receive data-graphing knowledge directly from the font, much in the way that Odin got his wisdom from Mimir. Maybe we wouldn’t need to give up an eye for it; but I don’t know, those Yalies don’t come cheap. Anyhow, you’re stuck with me. I’ve been using the MS Office triumvirate and assorted graphics programs since they were available for desktop computers. I’ve picked up a thing or two. Also, I do a good bit of art and science, which can be a beneficial pairing if you keep it under control. Yes, yes, I know, you got your Ph.D. at Cornell. We’re all very proud of you. But does it matter if no one can pick up the nuanced brilliance of your work? Take a few minutes and join us, won’t you?

CONTRIBUTED PAPERS. Wednesday, 2:00-4:00
BACTERIA 2
Contributed paper. Wednesday, 2:00. 135
Quantification of the dose of lepidopteran activity in new cotton events expressing the insecticidal protein Vip3A
David O’Reilly, Natalie Dupen, Janet Cairns, Kirsty Windle, Rhiannon Hughes, Mark Gill, Andy Blake, and Jacqui Sheridan
Syngenta, Jealotts Hill Research Center, Bracknell, Berks, RG42 6EY, UK

Vip3A is a novel insecticidal protein derived from Bacillus thuringiensis that is distinct from the Cry or Cyt toxins. It is active against a broad spectrum of lepidopteran pests. A series of transgenic cotton events that express Vip3A have been generated and are being prepared for commercialisation. The development of insect resistance is a key risk for all Bt cottons, and the US EPA has mandated a “high dose plus structured refuge” strategy to mitigate this risk. Five methods are recommended by the EPA that can be used to determine whether an event expresses a defined high dose of toxin against a particular pest species. Here, we present data from a selection of these methods investigating the high dose status of two Vip3A-expressing cotton events vs. the key target pests Helicoverpa zea (tobacco budworm) and Helicoverpa armigera (cotton bollworm).

Identification of vip3A-type genes from Bacillus thuringiensis strains and characterization of two novel vip3A-type genes
Jinhuan Liu1,2, Fuping Song1, Jie Zhang1, Jianxin Tan1
1State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, P. R. China, 2Agricultural University of HeBei, Baoding 071000, P. R. China

A PCR-RFLP strategy was used to rapidly identify Bacillus thuringiensis strains harbored the known or unknown vip3A-type genes. A pair of universal primers were designed based on conserved regions of vip3Aa and vip3Ab genes, and digested fragments of amplified products of 1456 bps with the Hind III and EcoRI enzymes were run on agarose gel to identify vip3A-type genes with different RFLP patterns. The vip3A gene-types of 607 B. thuringiensis strains were characterized and three kinds of RFLP patterns were successfully identified. 316 of them contained vip3Aa genes. 52 isolates including strain BTAI displayed a novel RFLP pattern of 876-bp, 260-bp, 160-bp and 160-bp fragments while 15 isolates including strain Btal had another new RFLP pattern of 1146-bp, 155-bp and 155-bp fragments. The full length of vip3A gene of BTAI containing 2409-bp was obtained and shared 96% sequence homology withvip3Af1 (isp3a) gene and 93% with vip3Aa12 (vip3A-WB5) gene. The novel gene was subcloned into vector pET-21b and overexpressed in E. coli BL21. The expressed protein was toxic to the Lepidoptera larvae. 1456-bp fragment of vip3A gene in BTAI was cloned by PCR method and it shared 83% sequence homology withvip3Af1 (isp3a) gene.

Contributed paper. Wednesday, 2:30. 137
Two novel classes of secreted insecticidal proteins of Bacillus thuringiensis
William P. Donovan1, James T. Engleman2, Judith C. Donovan2, William P. Clinton1, Oliver M. Ilagan1, Karina C. Krasomil-Osterfeld1, Thomas M. Malvar2, John W. Pitkin1, Matthew R. Walters1, James A. Baum1, and James K. Roberts1
1Monanto Company, St. Louis, MO 63017, 2Ecogen Incorporated, Langhome, PA 19047, USA

Bioassay screening demonstrated that culture supernatants of Bacillus thuringiensis strains EG2158 and EG5438 were toxic to Colorado potato beetle (CPB) larvae and diamondback moth (DBM) larvae, respectively. Ion exchange fractionation revealed that EG2158 produced a secreted protein of approximately 38 kDa that exhibited insecticidal activity to CPB. EG5438 produced a secreted protein of approximately 70 kDa that exhibited insecticidal activity to DBM. Gene specific oligonucleotides, whose designs were based on partial sequences of the purified ~38 kDa and ~70 kDa proteins, were used to clone the corresponding genes. The gene for the ~38 kDa protein encoded a protein of 367 residues (41,492 Da) whose sequence had no significant homology to any proteins in GenBank. The gene for the ~70 kDa protein encoded a protein of 601 residues (68,869 Da) whose sequence was distant related (49% identity) to the toxin portion of the B. thuringiensis CryICa protein.

Contributed paper. Wednesday, 2:45. 138
Enterotoxigenic genes: Are they involved in insecticidal activity in Bacillus thuringiensis?
George Kye-Poku, Debbie Gauthier and Kees van Frankenhuyzen
Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario P6A 2E5, Canada

Bacillus thuringiensis (Bt) is a gram-positive aerobic and facultative anaerobic endospore-forming bacterium found in the environment and is widely used as a biopesticide. B. thuringiensis belongs to the B. cereus group. Members of this group are known to produce emetic and enterotoxins that have been implicated as the causal agents for emetic and diarrhal syndromes. PCR and Southern hybridization were used to screen commercial B. thuringiensis products (Foray™ 48B, XenTari™ WD6, Vectobac™ and Novodor™) that are used in...
Canadian agriculture and forestry for the presence of known and putative *B. thuringiensis* virulence factors. With the exception of cytotoxin-Cry*F* gene, all the enterotoxin genes tested were present in the commercial products used in this study. In vivo expression of these genes is being evaluated in spruce budworm, *Choristoneura fumiferana*, gypsy moth, *Lymnastria dispar* and Colorado potato beetle, *Leptinotarsa decemlineata*. We will discuss the possible implications in deleting these virulence traits.

**STU** Contributed paper. Wednesday, 3:00.

**STU** Contributed paper. Wednesday, 3:15.

**STU** Contributed paper. Wednesday, 3:30.

**STU** Contributed paper. Wednesday, 3:45.

**STU** Contributed paper. Wednesday, 3:50.

**STU** Contributed paper. Wednesday, 4:00.

**STU** Contributed paper. Wednesday, 4:15.

**STU** Contributed paper. Wednesday, 4:30.

**STU** Contributed paper. Wednesday, 4:45.

**STU** Contributed paper. Wednesday, 5:00.

**STU** Contributed paper. Wednesday, 5:15.

**STU** Contributed paper. Wednesday, 5:30.

**STU** Contributed paper. Wednesday, 5:45.

**STU** Contributed paper. Wednesday, 6:00.

**STU** Contributed paper. Wednesday, 6:15.

**STU** Contributed paper. Wednesday, 6:30.

**STU** Contributed paper. Wednesday, 6:45.

**STU** Contributed paper. Wednesday, 7:00.

**VIRUSES**

**Poster / Viruses. V-1. Heterologous baculovirus pathogenicity in the absence of contemporary coevolution**

Gaétan Moreau¹, Christopher J. Lucarotti¹, Edward G. Ketteley¹, Kevin N. Barber², Stephen E. Holmes¹, Stephen B. Holmes², Charles Weaver¹, and Benoit Morin¹

¹Natural Resources Canada, Canadian Forest Service-Atlantic Forestry Centre, P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7, Canada, and ²Natural Resources Canada, Canadian Forest Service-Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie, Ontario, P6A 2E5, Canada

To examine the role of dynamic coevolution between baculoviruses and arthropod species in the maintenance of cross-infectivity, we fed a North American baculovirus to four Eurasian sawfly species. Our results did not support the hypothesis that contemporary coevolution is necessary for a baculovirus to maintain pathogenicity in a range of organisms as the ingestion of OBs from a North American baculovirus reduced larval survival by 51 to 82% in the four Eurasian sawfly species examined in the laboratory, and by 57% in the Eurasian sawfly examined in the field. Probing and microscopic examinations of dead larvae, as well as the absence of carry-over of baculoviruses, did not result in the transmission of OBs to naïve insects.
effects in field populations indicated that the inoculated sawfly species failed to produce OBs following exposure to the foreign baculovirus. This study might be the first field trial of a heterologous baculovirus against an exotic species in a forest ecosystem and to the best of our knowledge, baculoviral pathogenicity with abortive infection has never been reported in vivo in the laboratory or in field populations. We caution that special care should be taken with foreign baculoviruses introduced to control exotic pests, especially sawflies, because of their potential pathogenicity to non-target indigenous species.


Ecosystem alteration modifies the relative strengths of top-down and bottom-up forces in a herbivore population

Gaëtan Moreau1,2, Eldon S. Eveleigh1,2, Christopher J. Lucearoti1,2, and Dan T. Quiring1

1Natural Resources Canada, Canadian Forest Service - Atlantic Forestry Centre, P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7 Canada, and 2Population Ecology Group, Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, New Brunswick, E3B 6C2 Canada

Using exclusion techniques, we tested the hypothesis that alterations of the forest ecosystem associated with precommercial thinning have contributed to the increased severity of outbreaks of Neodiprion abietis (Harris), a sawfly defoliator, through the reduction of bottom-up (host plant) and top-down (natural enemies) trophic forces acting on N. abietis larvae. The relative contributions to N. abietis larval mortality of host-plant effects and a nucleopolyhedrovirus (NeabNPV) increased concurrently with increasing levels of defoliation and were both reduced by thinning. The reduction of the mortality associated with both host-plant and NeabNPV effects caused a 58% mean increase in N. abietis larval survival in thinned compared to untreated stands, which is less than would be expected by the sum of the effects of thinning on each mortality factor. Evidence indicates that the partly compensatory - partly additive nature of the mortality associated with host-plant effects and NeabNPV is responsible for this discrepancy. To the best of our knowledge, this is the first study to show the impact of ecosystem alterations on bottom-up and top-down forces acting on a terrestrial arthropod population, and how this can lead to increased outbreak severity.

Poster / Viruses. V-3.

Efficacy of indigenous TnSNPV and AcMNPV isolates for control of Trichoplusia ni: Greenhouse cage trials

Martin Erlandson1,2, Dave Gillespie1, Melissa Strom2, Don Quiring1, and David Thelma

1Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK, Canada, 2Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, SK, Canada, 3Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Agassiz and Summerland, BC, Canada

Indigenous strains of TnSNPV and AcMNPV were isolated from Trichoplusia ni, cabbage looper, populations infesting commercial greenhouse crops and the most active strain for each of TnSNPV and AcMNPV was selected based on laboratory bioassays. Feeding preference studies were conducted with 4th instar T. ni larvae and leaf discs of commercial cucumber, pepper and tomato varieties. Subsequently, dose uptake studies were conducted with 2nd and 4th instar larvae fed on potted plants of each crop species sprayed with either TnSNPV or AcMNPV at dose levels ranging from 10^8 to 10^12 PIB/ha equivalents. Interestingly, 4th instars showed higher rates of mortality in 7-day assays than did 2nd instar larvae. Crop plant also had a significant impact on virus-related mortality with pepper varieties typically having lower levels of cabbage looper mortality than other crops. Results from a preliminary greenhouse spray trial on cucumber indicate that on a per PIB basis AcMNPV was more efficacious than the TnSNPV isolate.


Relative activity of baculoviruses of the diamondback moth

Robert R. Farrar, Jr., 1 Martin Shapiro2 and B. Merle Shepard3

1USDA-ARS, Insect Biocontrol Laboratory, Beltsville, MD 20705, and 2Clemson University, Charleston, SC 29414, USA

The relative activities of the nucleopolyhedrovirus (PxMNPV) and the granulovirus (PxGV) of the diamondback moth against this insect were evaluated in the laboratory. Fewer occlusion bodies of PxMNPV were required to kill diamondback moth larvae, but much less PxGV-infected tissue was required to kill these larvae. Larvae that were killed by PxGV survived about 2 d longer than did those killed by PxMNPV. When diamondback moth larvae were fed both viruses together, mortality was slightly lower than would be expected from additive effects. Producing PxMNPV in an alternate host, the beet armyworm, did not reduce the potency of this virus against the diamondback moth. PxMNPV was less potent against the beet armyworm than was the beet armyworm nucleopolyhedrovirus.

Poster / Viruses. V-5.

Aerosol infectivity of baculovirus to insect larvae: A new larval inoculation strategy for baculovirus

Tzong-Yuan Wu1, Tyzy-Rong Jim2,3, Suey-Sheng Kao2 and Jason TC Tzeng1

1Department of Bioscience Technology, Chung Yuan Christian University, Chung Li, Taiwan, 2Biobicide Department, Taiwan Agriculture Chemicals and Toxic Substances Research Institute, Wufeng, Taiwan, 3Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan

The baculovirus-insect expression system is recognized as a popular recombinant protein production tool. The standard method to infect insect larvae with recombinant baculovirus for protein production is either by feeding occlusion bodies or by injecting the budded virus into the cuticle. In this report, we demonstrate that the recombinant baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) at titer above 10^8 pfu/ml can efficiently infect Trichoplusia ni (T. ni) larvae through aerosol inoculation of budded virus at 8 lb/in^2 pressure. This aerosol infection route for AcMNPV was restricted to T. ni and Plutella xylostella larvae, however, Spodoptera litura, and Helicoverpa armigera larvae appeared to be resistant to this aerosol inoculation process. This convenient baculovirus inoculation strategy can facilitate recombinant protein and virus production by insect larvae.


Fall armyworm Spodoptera frugiperda base line of susceptibility to baculovirus SNPV strain from Paraná, Brasil

Marcelino Vázquez G.1, Trinidad López P.1 and Jorge Vázquez R.2

1Laboratorio de Entomología, División de Ciencias Agronómicas, Universidad de Guadalajara, México, Las Agujas, Mpio. Zapopan, Jal. Km 15.5 Guadalajara-Nogales, 2División de Ciencias Biológicas, Universidad de Guadalajara, Las Agujas, Mpio. Zapopan, Jal. Km 15.5 Guadalajara-Nogales, México

Fall armyworm Spodoptera frugiperda is one of the most important pest of America and the most important corn pest in Mexico. Using entomopathogenic virus to control this species is now a potential alternative to suppress chemical pesticides in the field. For Spodoptera frugiperda in México there are no previous reports about use of baculovirus or potential of insect resistance to it. A population of Spodoptera frugiperda was obtained from a big collection made in Jalisco, México during July to September, 2004. A purified SNPV suspension from Embrapa, Brasil was used and the inclusion bodies (IBs) were determined. The number of IBs in the six 10 ml solutions (70, 700, 7000, 70000, 700000 and 7000000 IBs/10 ml) were used to impregnate 10 cm diameter discs of “castseed” leaves. Forty healthy third instar larvae were allowed to feed on discs for 24 hours. Cumulative mortality after 12 days was determined. The observed LC50 was 874 IBs between fiducial limits 182 and 2,662 and the regression line equation was y = 5.43 + 0.43(x - 13.93). Low slope indicates a wide range of mortality response that includes 5

logarithmic cycles of the doses. Data about artificial selection of at least two generations and evolution of LC50s will also be presented.

**RAPD-PCR fragments marking resistance and susceptibility of *Lymantria dispar* to nuclear polyhedrosis virus**  
Anatoly V. Ivashov1, Andrei P. Simchuk1, Vladimir V. Oberemok1 and Vladimir V. Gouli2

1Department of Ecology, V.I. Vernadsky National University, UA 95007, Ukraine, 2Entomology Research Laboratory Department of Plant and Soil Science, PO Box 53400, Burlington, VT 05405-34002, USA

For experiments the hundred gypsy moth larvae (3rd instar) were infected with NPV (“VIRIN-NSH” formulation) in dose of 104 inclusion bodies per larva. Fresh-perished larvae were immediately frozen. The experiment was terminated when insects reached 4th instar. Alive larvae were also frozen. Accumulation of the NVP was estimated per capita and per 1 mg of larval weights. DNA was extracted from head capsules of the larvae after extraction of the virus from their bodies. DNA extraction was realized in accordance with standard method (Sambrook et al., 1989). OPA-14 primer, TCTGTGCTGG (Operon Technologies, USA), was used for RAPD-PCR. Reaction was initiated in 25 mkl of the mixture with PCR reagents from GenePaKTM PCR Universal (IsoGen, Moscow) at the “Tercyc” amplificatory (DNA-Technology, Russia). Amplification was carried out as follows: 1 cycle of denaturation during 5 min at 95°C with following 45 cycles by the following scheme: 95°C (1 min), 56°C (1 min), 72°C (2 min). The reaction was terminated after 10 min at 72°C. Products of amplification were separated by electrophoresis in 1.8% agarose gel. DNA-markers M 100 (IsoGen, Moscow) were used as mass markers with lengths from 100 to 1000 nucleotide pairs. Application of OPA-14 primer to gypsy moth allows us to obtain 15 RAPD fragments in total. Electrophoresis spectrums of individual larvae may contain from 5 to 10 fragments. Two of 15 obtained fragments mark accumulation of NPV in gypsy moth larvae. Appearance of the PARD-fragment with length of 850 nucleotide pairs in larval spectrum coincides with low virus accumulation: 2530833 inclusions per capita against 17812071 inclusions per capita (t=2.1; d.f.=19; P=0.049). RAPD-marker with length of 430 nucleotide pairs show the same trend: 2510802 inclusions per capita against 178312017 inclusions per capita (t=2.23; d.f.=14; P=0.043).

These RAPD-fragments thus mark gypsy moth larvae resistance to NPV. Some RAPD-fragments were found to mark variation in diameter of inclusions and accumulation of NPV in gypsy moth larvae. DNA fragments with lengths of 1000, 850, 430 and 190 nucleotide pairs show statistically significant marking effect for low variation in diameter of inclusions and their accumulation in larval bodies. Fractions with lengths of 560 and 330 nucleotide pairs show opposite tendency. Low variations in studied parameters always coincide with their low mean values. These results may be interpreted as follows: appearance of this genetic material is related with stronger limitation in accumulation of NPV than other factors influence this process; its absence does not limit the process of the virus accumulation, but influence of other factors increase in its variability among the larvae. Thus, it may be concluded that fractions with lengths of 1000, 850, 430 and 190 nucleotide pairs mark the gypsy moth resistance to NPV while fractions with lengths of 560 and 330 nucleotide pairs mark the gypsy moth susceptibility to the virus.

Poster / Viruses. V-8.  
**Production of the *Lymantria dispar* nucleopolyhedrovirus in stirred tank bioreactors**  
James M. Slavicek and J. Matt Gabler

USDA Forest Service, Northeastern Research Station, Delaware, OH, USA

Production of LdMNPV in bioreactors could be less costly than producing the virus in insects, and would provide a clean product. We are developing methods for production of LdMNPV in 3, 7, and 14 liter stirred tank bioreactors as a prelude to production in 500 liter systems. A maximum cell density of approximately 1 x 10^7 Ld652Y cells/ml was achieved, when using an agitation speed of 75 rpm and a dissolved oxygen concentration of 50% of saturation. Analysis of spent medium revealed that no amino acid, vitamin, or glucose was depleted. A high utilization (from 27 to 38%) of aspartic acid, glutamine, tyrosine, and serine was found, and 54% of the riboflavin was used. Cholesterol may be limiting cell growth since it was depleted from the medium. There was no build up of the metabolic byproducts ammonia and lactate. Polyhedra production was found to be significantly greater when using a multiplicity of infection (m.o.i.) of 0.005 virus particles per cell compared to using an m.o.i. of 0.01 using the same starting cell density. Polyhedra production levels of 4.8 x10^11 and 5.8 x 10^11 polyhedra per liter have been achieved to date in the 3 and 7 liter bioreactors, respectively.

**In vitro propagation of NPVs from *Lymantria xylina***  
Chin-Tai Ku, Chih-Yu Wu and Chung-Hsiung Wang

Department of Entomology, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei 106, Taiwan, R.O.C.

The host plant of the casuaria moth, *Lymantria xylina* (Lepidoptera: Lymantriidae), has been expanded to more than 100 broadleaf trees species. The epizootic nucleopolyhedrosis of *L. xylina* larvae occurs frequently. IPLB LD-652Y cells and a new *L. xylina* cell line (NTU-LY2) were used to establish in vitro propagation of pathogens from moribund *L. xylina* larvae. Two NPVs (nucleopolyhedroviruses), LdNPV-like virus and LxyxNPV, had been found, these NPVs had been propagated in cell lines. Both NPV's showed 50-80% infection rate (PIB-forming cells/infected cells) to LD cells but LxyxNPV caused lysing effect to LY cells, only a few LY cells formed PIB. The LdNPV-like virus formed FP (1-2 polyhedra/cell) in both cells while LxyxNPV formed the condensed nuclei of the infected LD cells or a few infected LY cells, the condensed nuclei were filled with PIbs (MP). In fact, LY2 cells were persistently infected with LxyxNPV by PCR detection, the chronic infection transferred to acute infection in LY2 cells occurred frequently in the aged cells or malnutrition of cells. In this study, we confirmed that two NPVs, LdNPV-like virus and LxyxNPV, existed in Taiwan’s *L. xylina* populations and LY2 cells might be an available tool for studying the persistent infection of NPV.
Genetic modified baculovirus (GMBV) are among the most promising alternatives to chemical insecticides. One of the deterrents to the GMBV development is the lack of simple and cost-effective methods for monitoring their efficacy and ecology in fields. Here we demonstrate the DsRed gene from coral can serve as a convenient GMBV tracer. Insect larvae, including *Trichoplusia ni*, *Spodoptera exigua*, and *Spodoptera litura*, infected the GMBV containing the DsRed gene can emit red fluorescence under sun light without any prosthetic apparatus.

We previously reported that gypsy moth larvae grow more resistant to mortal baculovirus infection as they age within an instar, becoming most resistant during the actively feeding midgut of a given instar, with resistance diminishing partially as they prepare to molt to the next instar. This form of resistance is both midgut-based and systemic. We previously observed that mid-fourth instar (i.e., 48 hour post-molt) larvae that were starved for 24 or 48 hours prior to oral or intrahemocoelic challenge with LdNPV were just as sensitive to mortal viral infection as newly molted larvae. Recent experiments showed that starving larvae for as little as 3 hours prior to inoculation with occluded virus dramatically reduced their resistance to mortal infection; the relationship between increasing susceptibility and duration of starvation was linear up to 12 hours of starvation. After 12 hours without food, larvae were as sensitive to infection as newly molted 4th instars. We suggest that a population-level reduction of intrastadial developmental resistance to LdNPV due to starvation might partially explain the increased susceptibility of gypsy moths to LdNPV epizootics near the end of outbreaks when significant defoliation of host trees has occurred.

The GP64 protein of AcMNPV rescues HasSNPV transduction in mammalian cells

Changyong Liang1,2, Jianhua Song1,2, and Xinwen Chen1

1State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, People’s Republic of China, 2Graduate School of the Chinese Academy of Sciences, Beijing, 100039, People’s Republic of China

Group I nucleopolyhedrovirus (NPV), such as *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV), expresses the GP64 fusion protein that is essential for virus entry. However, Group II NPVs, such as *Helicoverpa armigera* nucleopolyhedrovirus (HasSNPV) and granuloviruses, do not express the gp64 protein but instead encode a different envelope protein called F. AcMNPV was shown to transduce a broad of mammalian cell types and express foreign genes under the control of mammalian promoter. It is particularly interesting that whether group II NPVs can transduct mammalian cells. We found that the group II NPV, HasSNPV encoding a different envelope protein F shows no detectable infectivity to mammalian cells, suggesting that the F protein cannot mediate baculovirus transfer into mammalian cells. This limitation was overcome by expressing the AcMNPV-GP64 in HasSNPV. Even with lower transduction ratios overall, the range of transduced mammalian cell types with the HasSNPV recombinant is consistent with AcMNPV. These findings indicate that the F protein functions only in insect cells whereas the GP64 protein works both in insect and mammalian cells.

A cellular Drosophila melanogaster protein with similarity to baculovirus envelope fusion proteins

Oliver Lung1 and Gary W. Blissard2

1Department of Biological Sciences, University of Lethbridge, Lethbridge AB, T1K 3M4, Canada, 2Boyce Thompson Institute, Cornell University, Ithaca, NY 14850, USA

F (fusion) proteins are found in the envelopes of the budded virions of baculoviruses. Recently an insect gene that encodes a protein with sequence similarity to F proteins was discovered (*Dm-cF*, short for *D. melanogaster* cellular F). We also identified genes related to *Dm-cF* in three other insects. To examine similarities and differences between insect and baculovirus F proteins, we cloned the *Dm-cF* gene, and analyzed 1) membrane fusion activity and cellular localization of transiently expressed Dm-cF in cultured cells; 2) *Dm-cF* expression in *D. melanogaster* of different developmental stages. Transiently expressed Dm-cF showed no protein cleavage, no detectable membrane fusion activity, and localized to intracellular organelles in S2 cells. In contrast, previously characterized baculovirus F proteins show localization to the plasma membrane of infected cells. RT-PCR and Western blot analysis of Dm-cF expression in flies showed that Dm-cF transcripts and protein were only present in 3rd instar larvae and adults, but not in earlier developmental stages. These studies suggest that Dm-cF expression is developmentally regulated and suggest that if baculovirus F genes are derived from a host cellular gene, the function and localization appears to have changed substantially upon adaptation to the baculovirus infection cycle.
STU Poster / Viruses. V-16.

Screening of cellular factors which interact with Host Range Factor-1 (HRF-1) from *Lymantria dispar* nucleopolyhedrovirus (LdMNPV)  

Hiroki Ishikawa1, Motoko Ikeda1, Suzanne M. Thiém1, and Michihiro Kobayashi1  

1Graduate School of Bioscience and Biotechnology, Nagoya University, 
Chikusa, Nagoya 464-8603, Japan.

**HRF-1, specifically encoded by LdMNPV genome, is an essential viral factor required for productive infection of NPV's in Ld652Y cells from L. dispar. NPV's infection induces global translation arrest in Ld652Y cells, and this arrest is precluded by HRF-1. However, the molecular mechanism by which HRF-1 precludes global translation arrest remains unknown. To identify cellular partners of HRF-1, a yeast two-hybrid screen of AD-fused cDNA library derived from Ld652Y cells was performed using BD-fused full-length HRF-1 as bait. We isolated 150 positive clones, 17 of which contained partial Ld652Y cells was performed using BD-fused full-length HRF-1 and infected with recombinant coimmunoprecipitation assay. Ld652Y cells were transfected with a plasmid expressing cmyc-fused eIF3a, and infected with recombinant HycuNPV that expressed HA-fused HRF-1. After immunoprecipitation using HA antibody, cmyc-eIF3a was coimmunoprecipitated with HA-HRF-1, indicating that HRF-1 can interact with eIF3a in NPV-infected Ld652Y cells. The potential role of the interaction of HRF-1 and eIF3a in NPV-infected Ld652Y cells will be discussed.**

Poster / Viruses. V-17.

Characterization of the *gp41* gene of *Spodoptera litura* multicapsid nucleopolyhedrovirus  

Lijing Pan, Zhaofei Li, Yingxue Gong, Mei Yu, Kai Yang and Yi Pang  

State Key Laboratory of Biocontrol, Zhongshan University, 
Guangzhou 510275, P.R. China

**Gp41 is one of the core genes among baculoviruses. *Spodoptera litura* multicapsid nucleopolyhedrovirus (SpltMNPV) gp41 gene is 993 bp long and encode a polypeptide with a predicted molecular weight of 36.89 kDa. Splt-gp41 transcripts were detected from 12 to 96 h post-infection (p.i.) and the mRNA start site was mapped within a consensus baculovirus late promoter sequence (ATAAG). Western blot analysis of extracts from SpltMNPV-infected *S. litura* cells detected a 41 kDa protein, and this protein was present in the nucleus of infected cells from 12 to 96 h p.i., whereas in the cytoplasm from 24 to 96 h.p.i. Structural localization confirmed that SIGP41 is associated with the envelope of occlusion derived virus (ODV). Glycosylation analysis of Splt-GP41 indicated that it is N-glycosylated. Lectin-binding assay showed that three lectins *Erythrina cristagalli* lectin (ECL), *Lycopersicon esculentum* lectin (LEL), and *Bandeiraea simlicifolia* lectin (BSL) recognizing N-acetylgalactosamine were specifically bound to SpltGP41. It was proposed that SIGP41 is an O-glycoprotein.**


Molecular cloning and functional characterization of a putative glycosyltransferase family 8 member *Lsp13* in *Leucania separata* multiple nuclear polyhedrosis virus  

Yingle Liu, Enqi Du, Huazhong Xiao, Weixin Jin, Yipeng Qi  

State Key laboratory of Virology, College of Life Science, Wuhan University, Wuhan 430072, People's Republic of China

**P13 is one style of specific gene in group II nuclear polyhedrosis virus. In this article, lsp13 was amplified from our reported LsMNPV genome (group II),which was an early and late expression gene in *Leucania separata* multiple nuclear polyhedrosis virus (LsMNPV) and encoded a putative glycosyltransferase family 8 member.**

However, it only showed very little homology with other members in their family in amino acid. In order to experimentally identify its possible functions on glycoprotein glycosylation and viral infection, we co-expressed Lsp13 with another LsMNPV glycoprotein Ld130 (F protein) and uniquely expressed Ld130 in s9 cells, respectively. HPLC results showed Ld130 molecular is larger than its uniquely expression. We speculated that Lsp13 may changed Ld130 glycosylation and further affected viral fusion in infection. Confocal microscopy showed that a GFP-tagged P13 was only localized in the s9 cells nuclear membrane while it located in BTT-In S1B1-(Hi5) cells both nuclear membrane and plasma membrane. Interestingly, we found P13 expression decrease virus replication in s9 while accelerate the Beat worm death after infection. The result suggest that P13 may play another important roles in viral replication and infection.**


Functional analysis of FP25K of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus  

Dong Wu1, Fei Deng2, Xiulan Sun1, Huailin Wang1, Li Yuan1, Just M. Vlak2, Zhihong Hu1  

1State Key Laboratory of Virology, Key Laboratory of Molecular Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China, 2Laboratory of Virology, Wageningen University, Wageningen PD 6709, The Netherlands.

**The fp25k gene of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HearNPV) was studied in this research. HearNPV fp25k gene transcription was found starting from about 18 hours post infection, and protein could be detected from the same time with antisera against FP25K. To study the function of HearNPV fp25k, a recombinant HearNPV (HaBacWD11) with an enhanced GFP gene replacing the fp25k was constructed using HaBacH8Z, a bacmid of HearNPV which lacks the polyhedrin gene. Growth curve analysis showed that HaBacWD11 produced higher titers of BVs than its wild-type counterpart HaBacH8Z-GFP. Electron microscopic analysis indicated that at the late stage of infection, the number of intranuclear enveloped nucleocapsids in HaBacWD11 infected cells was much less than that of HaBacH8Z-GFP. A rescue recombinant virus HaBacWD14 was constructed by re-introducing fp25k gene into HaBacWD11. The growth curve and electron microscopic analysis of the rescued recombinant confirmed the increase of BV yield and the decrease of the virion production in infected cells was the result of fp25k deletion. The expression of membrane fusion protein (HA133) and ODV-E66 were studied with the expression level of Ha133 and ODV-E66 were observed in HaSNPV FP25K mutant.**


Characterization of an AcMNPV without virions occluded in the polyhedra  

Lihua Wang and Xiao-Wen Cheng  

Department of Microbiology, Miami University, Oxford, Ohio 45056 USA

**Nucleopolyhedroviruses (NPV) need to have virions occluded in the polyhedra to persist in the environment and to infect susceptible insects *per os*. In an *Autographa californica* nucleopolyhedrovirus (AcMNPV) recombination experiment, a mutant AcMNPV containing no virions in the polyheda was isolated. This mutant named AcDef forms normal polyhedra, but polyhedra could not infect *Trichoplusia ni* larvae as 30,000 polyhedra/third instar larva. However, normal replication in SF-21 insect cell line occurred fine. AcDef in infected cells are infectious to *T. ni* larvae. A pair of primers upstream of *p26* and downstream of *p74* was used to amplify DNA in a PCR reaction to determine the missing sequence using AcDef DNA as a template by cloning and sequencing. Sequencing data showed p26 ORF and its associated putative promoter sequence are completely missing. This indicates p26 is not essential for viral replication. There is also 988 bp of deletion at the 3’ end of the p74 ORF, which represents half of the coding sequence. As p10 is**
sandwiched between p26 and p74, it is completely gone in the AcDef genome. Therefore there is a total of 2,136 bp deletion in the genome of AcDef compared to wild type AcMNPV.


Analysis of the temporal expression of *Trichoplusia ni* single nucleopolyhedrovirus genes following transfection of TsN5-B1 cells

Manuela van Munster1, Les Willis2, Miria Elias3, Martin Erlandson2, David Thielmann2, Roland Brousseau1 and Luke Masson1

1Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec H4P 2R2, Canada, Pacific Agri-Food Research Centre, 4200 Highway 97, Summerland, B.C. V0H 1Z0, Canada, 2Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, Saskatchewan and Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

*Trichoplusia ni* Single Nucleopolyhedrovirus (TsSNPV) is pathogenic to the cabbage looper (*Trichoplusia ni*), a serious pest of many economically important crops, and shows potential as a biocontrol agent. TsSNPV is a group II Nucleopolyhedrovirus, possesses a large 134,394 bp circular double-stranded DNA genome; and transcription, like that of other baculoviruses, is temporally regulated and divided into four classes: IE (immediate early), E (early), L (late) and VL (very late). In order to classify the viral genes, a viral DNA microarray was developed for analysis of the temporal transcription profiles of the 144 potential genes of TsSNPV. Specific 70-mer oligonucleotides covering both the 5' and 3' part of each gene were designed, synthesized and printed onto Corning UltraGAPS™ glass slides. A set of positive controls was included on each slide to allow normalization of data. Linearly amplified mRNA was synthesized from total RNA extracted at different time points from TsN5-B1 cells transfected with TsSNPV DNA. The amplicons obtained were hybridized onto the chip and microarray data normalization; statistical analysis and visualization were performed using GeneSpring software (Silicon Genetics). Different experimental approaches (one or two color experiments) and data analysis strategies were used to obtain temporal classification of known and unknown viral genes within the four classes are compared and presented.

Poster / Viruses. V-22.

Organization of the *Choristoneura occidentalis* granulovirus genome

Shannon R. Coppons, Hilary A.M. Lauzon, Peter J. Krell1, Basil M. Arif1

Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste. Marie ON, P6A 2E5 1Department of Microbiology, University of Guelph, Guelph, ON, N1G 2W1, Canada

*Choristoneura occidentalis* granulovirus (ChocGV), a baculovirus that infects the western spruce budworm, has been fully sequenced and analyzed. To date, ChocGV is the only GV identified containing a homologue to the apoptosis inhibitor protein P35/P49 (27.0%), followed by SplVN P49 (25.7%), suggesting that it may be a P49 homologue rather than P35 homologue. A P10 homologue was also found in chocr24 as it followed the general P10 model with a coiled coil region, rather than P35 homologue. A P10 homologue was also found in *SpltNPV* P49 (25.7%), suggesting that it may be a P49 homologue. The genome of ChocGV, a nucleopolyhedrovirus, has an overall G+C content of 39.1 % and contains 151 predicted open reading frames (ORFs). Typical baculovirus homologous regions and transcription, like that of other baculoviruses, is temporally regulated and divided into four classes: IE (immediate early), E (early), L (late) and V (very late). In order to classify the viral genes, a viral DNA microarray was developed for analysis of the temporal transcription profiles of the 144 potential genes of TsSNPV. Specific 70-mer oligonucleotides covering both the 5' and 3' part of each gene were designed, synthesized and printed onto Corning UltraGAPS™ glass slides. A set of positive controls was included on each slide to allow normalization of data. Linearly amplified mRNA was synthesized from total RNA extracted at different time points from TsN5-B1 cells transfected with TsSNPV DNA. The amplicons obtained were hybridized onto the chip and microarray data normalization; statistical analysis and visualization were performed using GeneSpring software (Silicon Genetics). Different experimental approaches (one or two color experiments) and data analysis strategies were used to obtain temporal classification of known and unknown viral genes within the four classes are compared and presented.

Poster / Viruses. V-22.

The genome sequence of *Chrysoideis chalcites* nucleopolyhedrovirus, a baculovirus with two DNA photolyase genes

Monique M. van Oers1, Marleen H.C. Abma-Henken’s1, Elisabeth A. Hermi1, Just M. Vlak1

1Laboratory of Virology, Wageningen University, Wageningen, the Netherlands, 2Greenomics, Plant Research International BV, Wageningen, the Netherlands, 3Department of Biological Sciences, Imperial College London, United Kingdom

The genome of ChocNPV, a nucleopolyhedrovirus recently isolated from *Chrysoideis chalcites* larvae, has a size of 149,622 base pairs, an overall G+C content of 39.1 % and contains 151 predicted open reading frames (ORFs). Typical baculovirus homologous regions (hrs) were not observed. ChocNPV belongs phylogenetically to group II NPVs, but in contrast to other group II NPVs encodes a proliferating cell nuclear antigen. Twenty-four unique baculovirus genes were identified, including a gene encoding a novel RING finger protein with a possible role in postviral DNA replication. Most remarkable is the presence of two ORFs, phr-1 and phr-2, which encode class II cyclobutane pyrimidine dimer (CPD) DNA photolyases, proteins with a predicted role in photo-reactivation of UV damaged DNA. The two phr genes share 45% identity at the amino acid level and have different putative promoter motifs. They form a monophyletic group with photolyases of (entomo-) pox viruses. The presence of a full complement of genes potentially involved in preventing mutagenic incorporations (dUTPase, lid138, rl1 and rr2), as well as two genes (phr-1 and phr-2) with a predicted role in UV damage repair, might play an important role in the ecology of this virus and may indicate that ChocNPV is uniquely protected against mutations.

Poster / Viruses. V-23.

The genome sequence of the *Mamestra brassicae* nucleopolyhedrovirus: An Old World virus compared with New World isolates

Sarah L. Turner1, John P. Burden1, Chimdi Ekeke1, Dawn Field1, Gareth Wilson1, Rosemary S. Hails1, Robert Feldman2 and Robert D. Possee1

1CEH Oxford, Mansfield Rd Oxford OX1 3SR, UK, 2SymBio Corporation, 1455 Adams Drive Suite 2124, Menlo Park, CA 94025 USA

We have sequenced the genome of *Mamestra brassicae* nucleopolyhedrovirus (MbMNPV). To date we have a single contig of 151,820 bp and are undertaking a PCR/sequencing approach to close a gap of about 1000 bp. The full genome will be about 153,000bp, which is smaller than *Mamestra configurata* NPV (MacoNPV-90/2, 155,060 bp and MacoNPV-96B, 158,482 bp). The MbMNPV genome has an average G+C content of 40.2%, which is similar to both MacoNPV genomes (MacoNPV90/2, 41.7%; MacoNPV-96B, 40%). 126 putative open reading frames (ORFs) of 50-150 nucleotides that have 95% amino acid identity) to those of MacoNPV-96B. This suggests MbMNPV is most closely related to MacoNPV-96B. One striking difference between these genomes is the absence of seven contiguous ORFs from MbMNPV that are present in MacoNPV-96B. This suggests MbMNPV is most closely related to MacoNPV-96B. One striking difference between these genomes is the absence of seven contiguous ORFs from MbMNPV that are present in MacoNPV-96B. This suggests MbMNPV is most closely related to MacoNPV-96B. Four of the seven ORFs are absent from the MacoNPV-90/2 genome. These four MacoNPV-96B ORFs were suggested to originate from the *Xestia c-nigram* granulovirus (XecnGV).


Potential horizontal transfer of an insect trypsin-like serine protease to *Neodiprion sertifer* NPV and *N. lecontei* NPV

Alejandra García-Maruniak1, Hilary A. M. Lauzon2, Basil M. Arif2, Christopher J. Lucarotti3 and James E. Maruniak1

1Entomology and Nematology Department, University of Florida, Bldg 970, Natural Area Dr., Gainesville, Fl 32611, USA, 2Canadian Forest Service, Great Lakes Forestry Center, Sault Ste. Marie, Ontario, P6A 2E5 Canada, 3Canadian Forest Service, Atlantic
In vivo cloning and comparative characterization of eleven distinct entomopoxviruses isolated from sympatric populations of *Adoxophyes honmai* and *Homona magnanima* (Lepidoptera: Tortricidae)


Department of Bioregulation and Biointeraction, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

Sympatic populations of *Homona magnanima* and *Adoxophyes honmai* were sampled in a tea field at Muziho, Tokyo, Japan, to investigate the prevalence of an entomopoxovirus (EPV). The EPV was recorded in both *H. magnanima* and *A. honmai* with prevalence of 55% and 39%, respectively. A restriction endonuclease analysis (REN) detected 106 and 37 variants of EPV from *H. magnanima* and *A. honmai*, respectively. Submolar bands were observed in the profiles of all of the variants, suggesting that these variants were a mixture of more than one genotype. Eleven EPV clones were obtained using an in vivo cloning method. The REN showed that all isolates had unique genotypes, but they shared many co-migrating fragments regardless of the insect species from which the isolates originated, indicating that they were variants of the same species. To evaluate infectivity and speed of killing, neonates of *H. magnanima* and *A. honmai* were inoculated with 10^4 or 10^5 spheroids/ml of each isolate. The EPV isolates significantly affected both the infection rate and survival time of the tested insects. Isolates that were virulent to *A. honmai* tended to be virulent to *H. magnanima* as well, and those that killed *A. honmai* quickly also killed *H. magnanima* quickly. However, no clear relationship was observed between infectivity and speed of killing.

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Multitemperature single-strand conformational polymorphism - a method for detection of minute changes in baculovirus genome

Boguslaw Szewczyk, Piotr Barski, Maria Paluszek, Liliana Hoyo-Carvajal, William Sihler, Agnieszka Brzozowska, Martin Lobo de Souza

1Department of Molecular Virology, Intercollegiate Faculty of Biotechnology of the University of Gdańsk and Medical University of Gdańsk, K. Napierskiego 30, 80-822 Gdańsk, Poland, 2A&A Biotechnology, Dairy and Apiary Research Institute, 80-801 Gdańsk, Poland, 3Instituto de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Medellín, Colombia, 4Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, 70707-900, Brasília, Brazil

Classical single-strand conformational polymorphism (SSCP) analysis is based on the observation that single-stranded DNA fragments attain a number of conformational forms which may be separated by electrophoresis in native polyacrylamide gels giving a characteristic pattern of electrophoretic bands. Even minute sequence changes (e.g. point mutations) may have significant effect on electrophoretic pattern of single-stranded DNA. Changes of gel temperature during electrophoresis increase the sensitivity of mutation detection in PCR products; this technique was named MSSCP (where M stands for “multitemperature”). We have applied this method modified in our laboratories for characterization of baculovirus DNA fragments. A series of degenerate primers were synthesized after the comparison of the polyhedrin gene sequences of over 20 baculoviruses. Two sets of oligonucleotides were used as universal primers in PCR reactions containing genomic DNA from an array of nucleopolyhedrosis viruses including these of *Autographa californica*, *Anticarsia gemmatalis*, *Spodoptera frugiperda*, *Lymantanria dispar*, *Lymantanria monacha* and many others. PCR products were denatured and subjected to MSSCP electrophoresis where, after silver staining, they gave ssDNA band patterns characteristic for each baculovirus species. This technique can be potentially applied to detect baculoviruses in insects collected in the field, as well as to plant tissues and the excretions or bodies of predators without need for sequencing the PCR products. Often MSSCP can be used not only for species determination but also as an indication of genomic variability which can be related to infectivity.

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Searching for a homologue of the *Mythimna separata* entomopoxvirus gene encoding the protein lethal to the endoparasitoid *Cotesia kariyai*


Department of Bioregulation and Biointeraction, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

When the gregarious endoparasitoid *Cotesia kariyai* (Hymenoptera: Braconidae) parasitizes *Mythimna separata* (Lepidoptera: Noctuidae) larvae infected with an entomopoxovirus (MyseEPV), the embryos and larvae of the parasitoid die inside the host. Previous studies showed that a toxic factor, lethal to *C. kariyai*, was present in virion-free plasma (VFP) from *M. separata* larvae infected with MyseEPV. A 28-kDa polypeptide was purified and named "Protein Lethal to *C. kariyai* (PLCK)". The PLCK gene was cloned and consisted of a 1,280-bp open reading frame. In this study, we used PCR with degenerate primers to search for homologous of the PLCK gene in nine entomopathogenic viruses: the Hawaiian (PsunGV-H) and Oregon (PsunGV-O) strains of *Pseudaletia unipuncta* granulovirus (GV), the *P. unipuncta* nucleopolyhedrovirus (NPV) hypertrophy strain (PsunNPV-H), *M. separata* NPV (MyseNPV), *Jesita c-nigrum* GV (XecnGV), *Helicoverpa armigera* GV (HearGV), *Spodoptera litura* GV (SpltGV), *Agrutis segetum* GV (AgseGV), and *Autographa californica* NPV (AcMNPV). The PCR analysis revealed that PsunGV-H, XecnGV, and HearGV possessed homologues of the PLCK gene. VFPs from larvae infected with PsunGV-H, XecnGV, and HearGV, and PsunNPV-H were toxic to the parasitoid larvae. Western blot analysis using a polyclonal antibody raised against PLCK revealed that VFPs from larvae infected with PsunGV-H and XecnGV reacted with the antibody.
Cotesia plutellae polydnavirus (CpBV) is obligate mutualistic insect virus found in parasitic wasp, Cotesia plutellae. Genomes of CpBV consist of several double-stranded, circular DNA molecules with variable size. In this study, we cloned CpBV genomic segments using plasmid capture system (PCS), and 29 different segments ranging from 0.1 to 25.5 kb were cloned. Among these, the complete nucleotide sequence of CpBV-S30 segment was determined and seven putative ORFs which showed similarities with known proteins were predicted. The promoter activities of these seven ORFs were investigated using baculovirus expression system and EGFP as reporter. While the ORF3002 promoter showed highest activity in transient expression, ORF3004 and ORF3006 promoter showed highest activity in insect cells and larvae, respectively in expression assay using recombinant baculoviruses. To improve the insecticidal activities of Autographa californica nucleopolyhedrovirus (AcNPV) by expressing AaIT under the control of these early promoters of CpBV-S30, recombinant AcNPVs, Ac3003ProAaIT, Ac3004ProAaIT, Ac3005ProAaIT and Ac3006ProAaIT expressing AaIT under the control of ORF3003, ORF3004, ORF3005 and ORF3006 promoter, respectively were constructed. Among these recombinant viruses, Ac3006ProAaIT showed highest insecticidal activity against 3rd instar larvae of Spodoptera exigua. These results suggested that early promoters from CpBV could be successfully applied to improve pathogenicity of baculoviruses.

Identification and application of P9, the most highly expressed gene of WSSV
Han-Ching Wang1, Chu-Fang Lo and Guang-Hsiung Kou
1Institute of Zoology, National Taiwan University, Taipei, Taiwan

White spot syndrome virus (WSSV), the type species of the genus Nimaviridae, is an enveloped, ellipsoid, large (>300kb) double-stranded DNA virus. Microarray analyses have shown that at least 89.5% (476/532) of the WSSV ORFs are expressed in the gill tissue of WSSV-infected shrimp (Penaeus monodon). Microarray and EST analysis of the mRNA profiles in WSSV-infected cells found three WSSV genes that were very highly expressed, and further showed that the mRNA if the WSSV P9 gene consistently has the highest copy number of all. At the protein level, 2D gel analysis and MS/MS protein identification showed that this nucleocapsid, tegument and envelope proteins of the shrimp white spot syndrome virus virion

Identification of the nucleocapsid, tegument and envelope proteins of the shrimp white spot syndrome virus virion
Jyh-Ming Tsai, Han-Ching Wang, Guang-Hsiung Kou and Chu-Fang Lo
Institute of Zoology, National Taiwan University, Taipei, Taiwan

The protein components of the white spot syndrome virus (WSSV) virion have been well established by proteomic methods, and 40 structural proteins are currently known. However, some details of the virus structure and assembly remain controversial, including the role of the major structural protein VP26. After isolating WSSV nucleocapsids by treatment with Triton X-100 and CsCl isopycnic equilibrium centrifugation, mass spectrometry identified only VP664 (the major nucleocapsid protein) and four other minor structural
proteins. Surprisingly, VP15 was not detected in this fraction. To locate the other structural proteins, intact WSSV virions were separated into four distinct fractions, envelope, envelope-tegument, tegument-nucleocapsid and nucleocapsid, using Triton X-100 in combination with various concentrations of NaCl. VP66 and four other structural proteins were confirmed as nucleocapsid proteins. VP26 was identified as a tegument protein. VP28 and VP19 were identified as envelope proteins. For the sixteen known proteins for which we have antibodies, protein location in the virion were confirmed by SDS-PAGE analysis and Western blotting, and in some cases also by immuno-electron microscopy.

**STU Poster / Viruses. V-35.**

**Identification of basal promoter and enhancer regions in an untranslated region of WSSV ie1**

Wang-Jing Liu, Chu-Fang Lo and Guang-Hsiung Kou

Institute of Zoology, National Taiwan University, Taipei, Taiwan

Some WSSV (white spot syndrome virus) promoters have been shown to be active in insect cells. However, although these genes exhibit one characteristic of IE (immediate early) genes (i.e. that they can be transcribed from a transcript that is initiated at a site separate from the major transcriptional start site). Two of these genes were identified as immediate early genes. In our previous study, cycloheximide was successfully used as an inhibitor to block de novo viral protein synthesis during WSSV infection and a WSSV IE gene (namely the gene for IE1) was identified. In a promoter activity assay in S9 insect cells using EGFP (enhanced green fluorescence protein) as a reporter, ie1 showed very strong promoter activity, producing higher EGFP signals than the insect Orgyia pseudotsugata multicapsid nuclear polyhedrosis virus (OpMNPV) ie2 promoter. Then in a dual luciferase promoter activity assay in S9 insect cells using firefly luciferase as a reporter, ie1 showed the strongest promoter activity of all the WSSV gene promoters that are reported to be active in insect cells. To further analyze the WSSV ie1 promoter, we constructed several deleted promoters, from which the basal promoter and an enhancer region were identified.

**MICROSPORIDIA & PROTOZOA**

**Poster / Microsporidia. MP-1.**

**The Eppendorf® - micromanipulator - a new technique for the quantitative separation of microsporidian spores for infection experiments**

Thomas Kolling1, Daniela Pilar ska2 and Andreas Linde1

1Fachhochschule Eberswalde, Dept. of Forestry, Applied Ecology Alfred-Moelker-Str. 1, 16225 Eberswalde, Germany, 2Institute of Zoology, Bulgarian Academy of Sciences, Blvd. Tzar Osvoboditel 1, Sofia 1000, Bulgaria

Micromanipulators translate, in a continuously selectable gear reduction, the hand movements of the operator directly, evenly and without any backlash to the tools. It has proved to be extremely successful in all branches of medicine and biology where micro-surgical, physiological, or chemical operations have to be performed on living organisms like oocytes, adherent cells, or plant cells. Typical applications are microinjection in adherent cells, transgenics, and ICSl. We used an Eppendorf® combination of PatchMan® and CellTram® devices with Eppendorf® CustomTips (Type IV), attached to a Leica® inverse microscope, and adapted this technique for the separation of entomopathogenic spores. Individual microsporidian spores can be located and imbibed into a micropipette through low pressure. This procedure can be performed and observed in real time by use of an inverse microscope. In several microsporidian genera, different spore types may be produced. The genus Vairimorpha is characterized by two types of environmental spores: The diplocarpyotic or Nosema-like spores, and the mononucleate octospores. The significance of these two spore types for the development and/or transmission of the microsporidia is so far unknown, mainly due to experimental difficulties concerning the separation of the spore types. By use of micromanipulation we were able to separate and collect single spores from a mixed spore suspension of Vairimorpha sp., isolated from the fat body of Lymantria dispar L. We obtained a pure sample of octospores which we use for infection experiments. The method and the results of the infection experiments will be presented. Micromanipulators offer a precise method for the exact dosage of spore suspensions and a new and promising method for the study of the function of different microsporidian spore types.

**Poster / Microsporidia. MP-2.**

**A microsporidium infecting the black vine weevil, Otiorhynchus sulcatus (F.) (Coleoptera: Curculionidae)**

Denny J. Bruck1 and Leellen Solter2

1USDA-ARS Horticultural Crops Research Laboratory, 3420 N.W. Orchard Ave., Corvallis, OR 97330, and 2Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61801, USA

The black vine weevil, Otiorhynchus sulcatus, is a severe pest of ornamental and small fruit crops throughout the world. We report the first observance of a microsporidian parasite from adult O. sulcatus weevils that were originally collected from an ornamental nursery operation in the Willamette Valley of Oregon, USA in the summer of 2003. The spore morphology is typical of Nosema-like microsporidia, but the vegetative forms appear to be mononucleate. The microsporidium only infects the fore-, mid- and hindgut of O. sulcatus, causing the gut to become opaque in heavily infected individuals. Subsequent studies to characterize the biology of this organism revealed that it is highly virulent to third instar O. sulcatus; 80-95% of individuals ingesting as few as 100 spores dying within 12-16 days. We failed to locate the microsporidium in the field when conducting an initial follow up survey of five O. sulcatus infested locations (ornamental nurseries and strawberry fields) in the spring of 2005, including the location of original infected population. Although one species, Nosema otiorth symi, was described from O. ligustici, the alfalfa snout beetle, in the Czech Republic in 1951, the description was incomplete and it is not known whether the isolate from O. sulcatus is the same species. Upon completing a full description of the microsporidium, we plan to continue our research efforts to determine its effect on the host population in the field, its prevalence in weevil populations in other areas of the USA, and its potential as a microbial control agent.

**Poster / Microsporidia. MP-3.**

**Nosema ceranae infection in Apis mellifera**

Wei-Fone Huang, Jing-Hao Jiang, Chung-Hsiung Wang

Department of Entomology, National Taiwan University, Taiwan

The Nosema disease is a worldwide honeybee epizootic disease and caused heavy economic losses in honeybee industry. It is considered that Nosema disease of the honeybee, Apis mellifera, was caused by the infection of Nosema apis, while Nosema disease of the oriental honeybee, A. cerana, was infected with N. ceranae. The Nosema disease also occurred in the A. mellifera hives that we kept in the NTU campus, the infected workers were mashed and the spores were purified. The rDNA of purified spores was sequenced and analyzed, the rDNA repeat unit organization is similar to that of N. apis (5′-SSUrRNA-ITS-LSU(rRNA-3′), and the total length is 3,832 bp. However, the SSUrRNA sequence shares 98% identity with that of N. ceranae, and shows this isolate from A. mellifera is closely related to N. ceranae rather than N. apis. The SSUrRNA, ITS, and LSU(rRNA sequences are comparatively low identities with those of N. apis (91, 52, and 89%, respectively), that further confirm this isolate is N. ceranae. In this study, we first carried out the complete rDNA sequence from N. ceranae and gave evidence that N. ceranae infects not only A. cerana but also A. mellifera.

**Poster / Microsporidia. MP-4.**

**Phylogenetic analysis of the Nosema spp. from cruciferous lepidopteran pests in Taiwan**

Ku Chin-Tai1, Ching-Chou Tseng2, and Chung-Hsiung Wang3

1Department of Entomology, National Taiwan University, Taipei 106, Taiwan, 2Division of Bio-Pesticide, Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Taichung, Taiwan
The microsporidia isolates were collected from moribund larvae of the cruciferous lepidopteran pests. *Nosema spodopterae*, two isolates (PX1 and PX2) from the diamondback moth, *Plutella xylostella*, and the *Nosema* type species, *N. bombycis* were for this study. A N. bombycis antiserum, a *N. bombycis*-specific primer pair and the SSU rRNA gene sequence were preliminarily determined. The results showed that PX1 and -2 belonged to the genus *Nosema* complex, PX1 was closely related to *N. bombycis*. The complete sequences of PX1 and -2 rRNA genes were carried out and the organizations of rRNA genes exhibited a typical *Nosema*-specific organization: 5'- LSU rRNA - ITS - SSU rRNA - tRNA (Glu) - tRNA (Val) - 3' / 5'- 25S rRNA - 5.8S rRNA - 18S rRNA - RPB1 - RPB2 - 3'. The t-α and β-tubulins and RPβ1 of PX1, PX2 and *N. spodopterae* were also sequenced. The identities of rRNAs, spacers, and three other genes were compared. These genes were up to 95% identity, while a high divergence occurred in spacer regions. Except SSUrRNA, other gene sequences were used to construct the phylogenetic trees, these four *Nosema* species are consistently grouped in a clade, but PX-1 isolate is closer to *N. bombycis* and *N. spodopterae* than PX-2. In conclusion, PX-1 may be a new subspecies of *N. bombycis* but PX-2 is a new species and named *N. platetiae*.

Poster / Microsporidia. MP-5.

Complete sequence and secondary structure of ribosomal RNA gene of the *Nosema* sp. C01

Ji-Young Choi1, Jong-Gill Kim1, Young-Chool Choi1, Won-Tae Kim1, Ha-Sik Sim1, Jan Wuysts2 and Yeon-Ho Je3

1Department of Sericulture and Entomology, National Institute of Agricultural Science and Technology, R.D.A. Suwon 441-100, Republic of Korea, 2Department of Plant Biology, Flander Interuniversity Institute for Biotechnology (VIB), Ghent University, Technologiepark 927, B9052 Ghent, Belgium, 3Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Republic of Korea

We present here for the first time the complete DNA sequence data (3779 bp, GenBank Accession No. AY383655) of the ribosomal RNA (rRNA) gene of the Lepidoptera-infecting microsporidia species, *Nosema* sp. C01. The SSU rRNA consists of 1236 bp which is much shorter than a typical prokaryotic SSU rRNA. The predicted secondary structure of SSU rRNA consists of a core (formed by 1, 2, and 31 helices) and 4 branches (formed by 1-21, 22-30, 32-48, and 49-50 helices) from the 5' end clockwise to the 3' end. The helices 10, 11, 18, 37, 43, 45 and 46 were missing. The LSU rRNA is greatly reduced in length (2506 bp). In LSU rRNA secondary structure of LSU rRNA, eleven hypervariable areas are shown and nine helices (B6, B7, B8, B14, B21, D5, E9, E15, and G5) are missing. B7-B9 and D4 helices can be used for taxonomic studies. The ITS region (37 bp), positioned between the SSU and LSU rRNA genes. The establishment of microsporidial rRNA sequences and their secondary structure might contribute to their somewhat limited taxonomic classification based on morphology.

**MICROBIAL CONTROL**

Poster / Microbial Control. MC-1.

**Intraguild interactions between *Verticillium lecanii* (Zimmermann) Viegas and *Aphiidoletes aphidimyza* (Diptera: Cecidomyiidae) as biological control agents of *Myzus persicae* (Homoptera: Aphididae)**

Patricia Jaramillo1, Bernard Roitberg1, Mark Goettel1, Dave Gillespie2

1Department of Biological Sciences, Simon Fraser University, Canada, 2Agriculture and Agri-Food Canada, Canada

Intraguild interactions occur between species that are competing for similar and limited resources. Biological control organisms, such as predators or parasitoids of the target species, have intraguild interactions that can either enhance or disrupt their effectiveness. For my research, I will use lab and greenhouse experiment to evaluate the intraguild interactions between *Aphiidoletes aphidimyza* (Diptera: Cecidomyiidae) and *Verticillium lecanii* (Zimmermann) Viegas a predator and entomopathogenic fungus of the green peach aphid, *Myzus persicae* (Homoptera: Aphididae). My results will give insight into the implications of the simultaneous use of these biological controls in the greenhouse system.

Poster / Microbial Control. MC-2.

**Effects of *Verticillium lecanii* (*Lecanicillium spp.*) against two-spotted spider mite, *Tetranychus urticae* and its natural enemy *Physothoros persimilis***

M. Koike1, T. Kodanna1, A. Kikuchi1, M. Okabe1, K. Kuramoti1 and Y. Saito2

1Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Hokkaido, Japan, 2Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Hokkaido, Japan

Effects of using entomopathogenic fungus, *Verticillium lecanii*, against two-spotted spider mite (*Tetranychus urticae*), and its natural enemy (*Physothoros persimilis*) were studied. Four isolates of *V. lecanii* (Vertealce, Mycotcal, A-2, B-2) were evaluated for their lethal effect, avoidance and behavioral change against *T. urticae* and *P. persimilis*. All isolates of *V. lecanii* showed pathogenicity against predator mite, but its effect was lower than that of spider mite when examined at relatively low humidity; RH 66%. Thus we examined synergetic ability using *V. lecanii* with predatory mite on the field colony. There were no significant differences among treatments. However, there were tendency of high suppressibility when we applied B-2 with predatory mite. These results indicated that *V. lecanii* could be the candidate for biological control agent, moreover they could use with predatory mite.

Poster / Microbial Control. MC-3.

**Factors that influence the desiccation tolerance and storage stability of blastospores of the entomopathogenic fungus *Paeclomyces fumosoroseus***

Mark A. Jackson1, Sophie Cliquet2, Selim Erhan3, and William J. Connick, Jr4

1USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL, 61604, USA, 2Laboratoire Universitaire de Microbiologie Appliquée de Quimper, 2, rue de l’université, Quimper 29000 France, 3Georgia-Pacific Corporation, Bedford Park, IL, USA, and 4(Retired) USDA, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA, 70124, USA

The survival of blastospores of *Paeclomyces fumosoroseus* during drying and storage was dependent on nutrient during growth, drying protocols and amendments, and storage conditions. The desiccation tolerance of blastospores of *P. fumosoroseus* was enhanced if the liquid production medium contained high concentrations of available nitrogen. During freeze-drying, sugars such as glucose, sucrose, and lactose improved desiccation tolerance while storage stability was enhanced by the addition of whole milk or bovine serum albumin. In air-drying experiments, the use of moist air (RH > 65%) during drying enhanced the storage stability of blastospores of *P. fumosoroseus*. Under appropriate air- or freeze-drying conditions, blastospore survival after drying is 70% - 90%. Storage for 1 year at -20°C resulted in no significant loss in blastospore viability for freeze-dried preparations. When air-dried *P. fumosoroseus* blastospores were stored under vacuum at 4°C, 1 year storage resulted in no loss in blastospore viability with only a 40% loss in viability after 2 years storage. These studies have shown that liquid culture-produced blastospores of *P. fumosoroseus* can survive drying and remain viable during long-term storage if appropriate conditions are used in the production and stabilization of these propagules.
In 1999, an epizootic of *Metarhizium anisopliae* in a field population of wireworms (*Agritos obscurus*) on the southwest coast of Canada was exploited and isolates were acquired for experimentation. Other acquired isolates included F52 and several from field populations of *A. lineatus*. When screened against three species of wireworms (*A. obscurus, A. lineatus* and *Ctenicera pruinina*) under laboratory conditions, differential mortality responses were observed. Even though bioassays using as little as $10^3$ conidia/gram soil produced 100% mortality in many cases, the length of time wireworms were exposed to conidia-treated soil, soil temperature, and soil moisture played a large role in determining the degree of infection and mortality. Conidia were shown to have a repellent effect which increased from $10^3$ to $10^6$ conidia/gram soil, but the effect was reduced when a food source was present. Despite this, wireworms were not deterred from feeding on potato tubers dusted with conidia, which led to 73% mortality attributed to *Metarhizium infection* after 25 days in a simulated field experiment.

**Poster / Microbial Control. MC-5.**

**Virulence of fungal biocontrol agent *Beauveria bassiana* to the eggs and adults of carmine spider mite *Tetranychus cinnabarinus***

Wei-Bing Shi1 and Ming-Guang Feng1,2

1Institute of Applied Entomology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China, 2Institute of Applied Entomology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China

The fungal biocontrol agent *Beauveria bassiana* SGS8702 was bioassayed for its virulence to the eggs and female adults of carmine spider mite *Tetranychus cinnabarinus* at 25°C under a photophase of 12:12 (L:D). Exposures of the mite eggs (on *Vicia faba var. minor* leaves) to fungal sprays of 4, 8, 58, 298 and 1306 conidia/mm² resulted in corrected egg mortalities of 4.9, 9.3, 21.3, 36.5 and 65.0%, respectively. Infected eggs on the leaves failed to hatch and had fungal outgrowths when maintained under moist conditions. Exposures of female adults to the sprays of 39, 74, 172, 533 and 849 conidia/mm² caused the mortalities of 21.8, 39.4, 61.1, 77.6 and 97.2%, respectively. Natural mortalities of the eggs and adults were only 6.9 and 10.1% in blank controls. Adult bioassay data fit very well to time-concentration-mortality model, yielding the LC 50 only 6.9 and 10.1% in blank controls. Adult bioassay data fit very well to time-concentration-mortality model, yielding the LC 50 only 6.9 and 10.1% in blank controls.

**Poster / Microbial Control. MC-6.**

**Development of *Beauveria bassiana*-based mycoinsecticide for tea leafhopper control in China: Current status and prospects**

Ming-Guang Feng1,2 and Shen-Hua Ying1

1Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China, 2Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China

To develop a mycoinsecticide as an alternative for control of false-eye leafhopper *Empoasca vitis* infesting tea gardens in China, aerial conidia of *Beauveria bassiana* SGS8702 produced on low-quality rice, harvested by a machine “MycoHarvester” and vacuum-dried at ambient temperature were suspended in a mineral oil containing emulsifier, suspension stabilizer and UV protectant, forming an emulsifiable formulation of 1x10^6 or 2x10^6 conidia/ml. Inclusion of imidacloprid into the formulation at ~10% of its labeled rate is optional upon expected leafhopper control and tea quality. Pilot field trials were first conducted in Yunnan and Zhejiang provinces during 2001–2003 to determine the effect of application rates and methods on the efficacy of the fungal formulation against tea leafhoppers. Optimized application method was the low-volume spray of 100-fold aqueous dilution (150 L/ha) by a knapsack airblast sprayer with 1.6-horsepower gasoline engine. With this method, combined application of 1.5x10^10 and 3.0x10^10 conidia/ha with imidacloprid a.i. 4.5 g/ha yielded overall mean efficiencies of 70 and 86% in Zhejiang during summer and autumn 2003 respectively. Under supervision by authorized pesticide management agency in China, the fungal formulation successfully passed pre-registration field trials conducted independently in Hubei, Hunan, Jiangxi and Zhejiang provinces during 2003-2004 and all toxicological and/or pathological tests in authorized medical agencies. The fungal formulation will be an optional practice for tea leafhopper control in the near future.

**Poster / Microbial Control. MC-7.**

**Modeling analysis of the interaction of *Beauveria bassiana* and imidacloprid on two aphid pests**

Su-Dan Ye1 and Ming-Guang Feng1,2

1Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China, 2Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China

Application of chemical insecticides for control of the two aphid species *Myzus persicae* (Mp) and * Macrosiphoniella sanborni* (Ms) on either vegetables or chrysanthemum in southern China has been strictly restricted due to residue concerns. To search for alternative measures against the pests, a series of laboratory bioassays consisting of low, median and high conidial concentrations of *Beauveria bassiana* SGS8702 plus an increasing rate of imidacloprid were conducted to quantify the fungal/chemical interaction on Ms (0.01-0.05 μg/ml) and Mp (0.05-0.5 μg/ml). Ms was either more susceptible to *B. bassiana* or more sensitive to imidacloprid than Mp after exposure to 1-ml spray on 95-cm² area. Based on the time-concentration-mortality modeling, the fungal and chemical interaction depended on both concentration and post-spray time. Adding imidacloprid to fungal sprays at the rates of 0.025-0.05 μg/ml against Ms or 0.1-0.5 μg/ml against Mp significantly enhanced or accelerated the fungal action. The relative potencies of an imidacloprid-inclusive bioassay over those with *B. bassiana* alone or together with a lower chemical rate ranged from a few to hundreds of times and varied over days after spray. Thus, a combined formulation or application of *B. bassiana* and imidacloprid could be of practical value for aphid control.

**Poster / Microbial Control. MC-8.**

**Quantified interaction of fungal biocontrol agent *Beauveria bassiana* and a thioulat-diaminimium insecticide on *Plutella xylostella* larvae**

Li Tian1 and Ming-Guang Feng1,2

1Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China, 2Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310029, P. R. China

Tier bioassays were performed to quantify interaction between fungal biocontrol agent *Beauveria bassiana* SGS8702 and dimethylpyrazine, a thioulat-diaminimium insecticide, against diamondback moth *Plutella xylostella*. Second instar larvae were exposed to sprays of *B. bassiana* alone at the concentrations of 21-38, 157-232 and 822-1133 conidia/mm² or together with the chemical rates of 5, 10, 25, 50 and 100 μg/ml, respectively, and maintained at 25°C and 12:12 L:D for 8-day mortality observations. The modeling of the resultant time-concentration-mortality data indicates high virulence of the fungal agent to *P. xylostella* with the LC₅₀ being 269 conidia/mm² on day 4 and dropping to 107 on day 8. Lower lethal concentrations or shorter median lethal times resulted from fungal sprays including dimethylpyrazine at the tested rates, which never caused higher mortalities than the fungus alone. Fungal action over 3-7 days after spray was significantly enhanced by incorporating the dimethylpyrazine rates of
>25 μg/ml into the fungal sprays for LC_{50} decreases of 2.6-1756 folds, ≥50 μg/ml for LC_{50} decreases of 4-274 folds and 100 μg/ml for LC_{50} decreases of 9-33 folds. These rates were equivalent to 5-20% of the dimethyl rate labeled for field application. The fungal/chemical interaction highlights the feasibility of combined formulation or application of B. bassiana and dimethyl for P. xyllostela control.

Poster / Microbial Control. MC-9.

Susceptibility of larval stages of the aphid parasitoids *Aphis colemani* and *A. matricariae* to the entomopathogenic fungus *Beauveria bassiana*

Melanie Filotas^1^, John Sanderson^1^, Stephen Wraight^2^

^1^Department of Entomology, Cornell University, Ithaca, NY, USA, ^2^USDA Agriculture Research Service, US Plant, Soil, & Nutrition Laboratory, Tower Road, Ithaca, NY, USA

The green peach aphid, *Myzus persicae*, and the melon aphid, *Aphis gossypii*, are common pests of commercial greenhouse crops throughout the United States. The Hymenopteran parasitoids *Aphis colemani* and *A. matricariae* are commonly used for biological control of these pests, however their compatibility with the *Beauveria bassiana*-based Botanigard, the only microbial insecticide commercially available for use against aphids in US greenhouses, has not been fully investigated. While some studies have shown a high level of susceptibility of *A. colemani* to other *B. bassiana*-based products, we observed few negative effects on a natural population of *A. matricariae* when Botanigard was applied to green peach and melon aphids on potted chrysanthemum in the greenhouse. We are currently conducting a series of laboratory assays investigating susceptibility of larval stages of *A. colemani* and *A. matricariae* to sprays of Botanigard applied simultaneously and at varying intervals after parasitization of nymphal green peach and melon aphids. Mortality and mycoses will also be measured for nymphs exposed to sprays of Botanigard alone to investigate impact of parasitization on susceptibility to fungal infection.

Poster / Microbial Control. MC-10.

Compatibility and potential synergism between the entomopathogenic fungus *Beauveria bassiana* and the insect growth regulator azadirachtin for control of the greenhouse pests *Myzus persicae* and *Aphis gossypii*

Melanie Filotas^1^, John Sanderson^1^ and Stephen Wraight^2^

^1^Department of Entomology, Cornell University, Ithaca, NY, USA, ^2^USDA Agriculture Research Service, US Plant, Soil, & Nutrition Laboratory, Tower Road, Ithaca, NY, USA

One factor limiting the ability of entomopathogenic fungi to control rapidly developing insects such as aphids is the fact that frequent molting allows them to effectively remove fungal propagules before they can penetrate the cuticle, thus dramatically reducing their susceptibility to infection. One possible means of overcoming this is the use of fungi in combination with low doses of an insect growth regulator, which interfere with molting and thus may provide fungal propagules with more time to penetrate the host. We investigated this potential synergism and general compatibility between the neem-based insect growth regulator azadirachtin and the commercial *Beauveria bassiana* strain GHA against the two most common aphid pests of US greenhouses, the green peach aphid and the melon aphid. In a series of laboratory assays, *B. bassiana* was applied to first instar nymphs of each aphid alone, in combination with, and 1, 24, 48 and 72 hrs after application of azadirachtin. Preliminary results suggest that all combinations of *Beauveria* and azadirachtin caused higher levels of infection than did application of fungal spores alone, although effects were more pronounced for the melon aphid.

Poster / Microbial Control. MC-11.

Toxicity analysis of truncated insecticidal crystal proteins Cry1Ba3 from *Bacillus thuringiensis*

Guangjun Wang^1^, Jie Zhang^1^, Jun Wu^1^, Fuping Song^1^ and Dafang Huang^2^

^1^State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, P. R. China, ^2^Institute of Biotecnology Research, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China

To determine the minimal active fragment of Cry1Ba3 protein, six fragments of *cry1Ba3* gene with different length were amplified using PCR with different specific primers. These fragments were cloned into pET-21b vector at BamHI and SalI restriction enzyme sites, and then introduced into *E. coli* BL21 strain. After induced with IPTG, the proteins expressed by different fragments were analyzed by SDS-PAGE, and the result showed that all these truncated proteins could be expressed in *E. coli* BL21. Bioassay result showed that the truncated protein containing amino acids 1-685 and that containing amino acids 22-655 were highly toxic against *Plutella xylostella*, compared with full-length Cry1Ba3 protein, with LC_{50} 0.45μg/ml and 0.30μg/ml respectively; while peptides that consists of amino acids 85-655 and that consists of amino acids 22-627 lost their activities against *P. xylostella* completely; and the activity of truncated protein that consists of amino acids 54-655 was declined significantly, with LC_{50} 32.0μg/ml. Thus the minimal active fragment was located at N-terminal of Cry1Ba3 between position 22 and 655 and this result is helpful for its use in transgenic plant.

Poster / Microbial Control. MC-12.

Is phenoloxidase involved in induced resistance to *Bacillus thuringiensis* kurstaki in *Trichoplusia ni*?

Alida F. Jasmann^1^, Jessamy Mannson^1^, Jerry Ericsson^1^, Valérie Canion^1^ and Judith H. Myers^1^

^1^Department of Biological Sciences, 8888 University Drive, Simon Fraser University, Burnaby, BC V5A 1S6, Canada, ^2^Department of Zoology, 25 Harbord St., University of Toronto, Toronto, Ontario M5S 3G5, Canada, ^3^Department of Zoology, 6270 University Blvd., University of British Columbia, Vancouver, BC V6T 1Z4, Canada

*Trichoplusia ni*, a pest of greenhouse crops in British Columbia, has developed significant resistance to *Bacillus thuringiensis* kurstaki (Btk). In the laboratory, resistance in *T. ni* populations rapidly declines in the absence of selection which may be due to the presence of an inducible resistance trait. Individuals from 1) a Btk resistant population, 2) a population that had reverted from resistance to susceptibility and 3) a long-term susceptible, laboratory population were exposed to a sublethal Btk dose for 24 hours, and 3) larvae continually exposed to a sublethal Btk dose for one week. Hemolymph of larvae from the reverted-susceptible population continually exposed to Btk showed elevated phenoloxidase activity relative to naive larvae. Hemolymph protein concentration of the reverted susceptible larvae declined in proportion to the length of the Btk exposure period, whereas no changes in phenoloxidase activity or hemolymph protein concentration were observed in resistant populations from any exposure period.

Poster / Microbial Control. MC-13.

Construction of a *Bacillus thuringiensis* BAC library and partially cloning of *zitwtermicin A* biosynthesis cluster

Tiemei Shao^1,2^, Fuping Song^1^, Jie Zhang^1^, Daquan Liu^1^, Dafang Huang^1^, 2

^1^State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, P. R. China, ^2^Institute of Biotecnology Research, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China

*Bacillus thuringiensis* (B. thuringiensis) is well known and widely used as a biopesticide. To facilitate genome studies and to clone complex loci of this bacterium, a BAC library in *Escherichia coli* DH10B from genomic DNA of *B. thuringiensis* strain G30 was constructed. G30 is highly toxic to Lepidoptera like *Spodoptera*
exigua (hubner), Helicoverpa armigera (hubner) and inhibited the growth of Erwinia herbicola. The library, which contains 4713 clones with an average DNA insert size of 73kb (range from 5 to 162kb), representing approximately 59 equivalents of B. thuringiensis genome, which is the first reported BAC library from B. thuringiensis. The utility of this library was demonstrated through PCR screening of zwittermic A biosynthesis cluster in a 1000-clone subset. Four positive clones with different inserts were obtained. The sequence of zwittermic A biosynthesis cluster, which has been reported, was included in two positive clones and the inserts are about 65kb and 94kb. One positive plasmid was subcloned into the vector pBluescript sk(+) and partial sequence was obtained.

Implementation of the largest worldwide laboratory production of a baculovirus: The case of the nucleopolyhedrovirus of Anticarsia gemmatalis (Lep.: Noctuidae) in Brazil

Flavio Moscardi1 and Braulio Santos2

1Embrapa Soja, C. postal 231, 86001-970, Londrina, PR, Brazil
2Universidade Federal do Parana, Curitiba, PR, Brazil

The nucleopolyhedrovirus of A. gemmatalis (AgMNPV) is being employed annually on about 2.0 million ha of soybean in Brazil. Up to 2003 all production were made in the field for processing and formulation by private companies. Improvements on laboratory production procedures, presented in SIP 2003, resulted in a final product of much better quality and at a cost competitive with those of chemical insecticides. The procedures were further adjusted through the implementation in one of the companies (COODETEC) of a “Pilot Laboratory” for AgMNPV production (presented in SIP 2004). We now report the implementation of laboratory facilities at COODETEC for commercial production of the AgMNPV. These were inaugurated in November 2004 and consist of two independent laboratories: one for continuous insect production (700 m2) and another for virus production (750 m2). In the former, the insects are reared up adults, for egg collection and rearing the larvae up to the fourth instar in 500-ml cardboard cups (avg. of 300-350 larvae/cup). Daily, 3% of these larvae are placed in plastic boxes (30x35x12cm) containing diet and vermiculite for obtaining pupae to maintain the colony. The remaining larvae (97%) are transferred to the virus production laboratory, where they are placed in plastic boxes containing AgMNPV contaminated diet. Dead larvae are collected, processed and formulated as a bioinsecticide (COOPERVIRUS PM). COODETEC is expanding its AgMNPV production to reach its maximum capacity, which will involve 45 employees and inoculation of 600,000 larvae/day to produce virus to treat approximately 1.4 million hectares of soybean/year.

Laboratory and orchard studies on the transmission of Cydia pomonella granulovirus by contaminated C. pomonella adults

D. Winstanley1, J. V. Cross2, N. Naish3, G. Keane3, S. Hilton1, D. Gajek2, R. van Wezel2

1Warwick HRI, Wellesbourne, Warwick CV35 9EF, UK
2East Malling Research, West Malling, Kent, ME19 6BJ, UK
3University of Illinois, Laboratory for Entomological Research, Urbana, IL 61801, USA

Two formulations of C. pomonella granulovirus (CpGV) were assessed for their ability to be picked up by moths and transmitted to other moths by physical contact and during mating, as well as to the next generation via egg contamination. Several strategies were used including the use of fluorescent dyes to label the formulation, spread of infectious virus, scanning electron microscopy of contaminated adults and the use of a recombinant CpGV expressing the egfp gene behind the Drosophila hsp to detect low levels of infection in larvae. Results showing transmission of virus from adult to adult and from adult to offspring will be presented.

Evaluation of a native Heterorhabditis species from the Coastal Region of Central Perú against white grubs

J. Alcázar1, C. Farfán2, J. Salazar1, C. Castillo1, and H. K. Kaya1

1International Potato Center, Lima, Perú, 2Instituto Valle Grande, Cañete, Perú, 3Department of Nematology, University of California, Davis, CA 95616

The Cañete Valley in the subtropical Coastal Region of Central Perú is the most important area for sweet potato production. In the past few years, white grubs (Bothynix maimon and Anomala spp.) have become key pests of this crop. The larvae cause serious damage to the roots and adversely affect the quality, value and export possibilities of sweet potato. We conducted a survey for natural enemies of the white grubs and report the first isolation of an entomopathogenic nematode, a Heterorhabditis species, from the Peruvian Coastal Region. The nematode was isolated from larvae of B. maimon in corn and sweet potato fields in Cañete Valley at an altitude of 90 m. The LC50 of this nematode against white grub larvae (third instar Anomala sp.) in the laboratory was124 infective juveniles/larva, and a rate of 50 infective juveniles/cm2 caused 68% larval mortality in pot tests in the screen house. This nematode species was also efficacious against other pest insects including the sweet potato weevil (Euscepes postfasciatus) and two species of potato tuber moths (Phthorimaea operculella and Symmorischma tangolius).

Targeting the Andean weevils with a native entomopathogenic nematode species

S. Parsa1, J. Alcázar2, L. Lizarra2, and H. K. Kaya1

1Department of Nematology, University of California, Davis, CA 95616, USA, 2International Potato Center, Lima, Peru, CRIBA, University of Cusco, Cusco, Peru

An entomopathogenic nematode in the genus Heterorhabditis, designated as Alcazar-1, was isolated from potato weevil (Premnotrypes saturasculus) larvae and pupae at a potato storage shed in Huasahuasi, Peru. This entomopathogenic nematode was the first one isolated from Peru and shows potential as a biological control agent against weevil pests attacking Andean tubers. Besides potato, another Andean tuber grown by subsistence farmers is oca (Oxalis tuberosa) which serves as an important food source during the winter months. However, the oca weevil (Adioristidius tuberculatus) is a major pest of oca. Our objectives in this study were to determine the susceptibility of oca and potato weevils to Alcazar-1 and ascertain the potential of nematode dips to disinfest oca tuber seeds of weevil larvae and pupae. Our findings show that the Alcazar-1 is highly effective against the fourth-instar oca and potato weevil, can reduce infestation by the first-instar potato weevil larvae in seed potato in soil and can infect oca weevil larvae and pupae within a tuber when the tuber is dipped into a suspension of infective juveniles.

Virulence of various commercial isolates of Heterorhabditis bacteriophora against the European chafer (Rhizotrogus majalis)

Louis Simard1, Guy Bélair2 and Julie Dionne3

1Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, J3B 3E6, Canada
2Royal Canadian Golf Association, Golf House, Oakville, Ontario L6M 4X7, Canada
3University of Cusco, Cusco, Peru

The European chafer (EC) is the most damaging white grub species in Quebec. Heterorhabditis bacteriophora is widely recommended and applied for EC control on turf in eastern Canada. Our objective was to compare the virulence of five commercial isolates of H. bacteriophora against an EC population from Quebec. In the laboratory, four separated trials against the third-instar EC grubs were performed in 30-cm3 plastic Solo cups at 24°C. Nematodes were applied on a sandy soil at 0, 600, and 2000 IJs/larva/cup rates. After a 4-day exposure time, mortality was recorded on day 4, 6 and 10. In a greenhouse trial, nematode treatments were performed in plastic trays
established with Kentucky bluegrass (1-month growth) on a sandy soil. Nematodes were applied at the single rate of 16,000 IJs/30 larvae/tray (1.5 billion IJs/ha) and insect mortality was recorded 20 days post-treatment. In the laboratory, highly variable mortality rates were recorded between trials and between commercial isolates, ranging from 0 to 47%. With most isolates, no significant increase in mortality was recorded when increasing the nematode rate from 600 to 2000 IJs. In the greenhouse trial, mortality rates caused by H. bacteriophora were very similar to the ones recorded in the cups, ranging from 19 to 42%.

Poster / Nematodes. N-4

Entomopathogenic nematode production enhancement using physical and chemical host stressors
Ian M. Brown1, David I. Shapiro-Ilan2 and Randy Gaugler2

1Biology, Georgia Southwestern State University, Americus, GA 31709, USA, 2USDA-ARS, SE Fruit and Tree Nut Research Lab, 21 Dunbar Rd, Byron, GA 31008, USA, 3Entomology, Rutgers University, 93 Lipman Drive, New Brunswick, NJ 08901, USA

Elevated stress may compromise a host insect’s defenses, thus increasing its vulnerability to parasitism or disease. Entomopathogenic nematode infection may be enhanced for in vivo production purposes by stressing the host with physical and chemical agents. The enhancement of Heterorhabditis bacteriophora (hb strain) infection of Tenebrio molitor larvae was examined using the following stressors, temperature extremes, insecticidal oils, metal ions Mg2+ and Mn2+ and the insecticide imidacloprid. Dry heat (40°C for 30 mins) and hot water (60-70°C for 1 sec) significantly increased H. bacteriophora infectivity with respect to the unstressed control. Infective juveniles were assessed for all stressors that caused greater than 80% larval infection. No stressor tested was found to significantly reduce yields compared to the controls. The cation Mn2+ was the only chemical stressor to enhance infectivity compared to the control. Most chemical stressors caused high host mortality and low infectivity rates. We conclude that induced host stress has the potential to enhance entomopathogenic nematode infectivity for in vivo production.

Poster / Nematodes. N-5

Infectivity of entomopathogenic nematodes and immune responses of their insect hosts
Xinyi Li1, Richard S. Cowles2, Elizabeth Cowles3, Randy Gaugler4 and Diana L. Cox-Foster4

1Department of Entomology, The Pennsylvania State University, University Park, PA 16802, 2Valley Laboratory, The Connecticut Agricultural Experiment Station, Windsor, CT 06095, 3Department of Biology, Eastern Connecticut State University, Willimantic, Connecticut 06226, 4Department of Entomology, The Rutgers University, New Brunswick, NJ 08901, USA

Entomopathogenic nematodes (EPNs) are ecologically and economically important. Two families of insect EPNs are good candidates of biological-control agents, but they do not significantly reduce pesticide application because of their poor host suitability. Infective juveniles (IJ) of EPNs penetrate insect hosts and release symbiotic bacteria that kill the insect hosts and serve as food resources for EPNs. Insect hosts defend against EPNs by a rapid cellular immune response resulting in encapsulation and melanization that kills EPNs. The nematodes have to overcome the innate immunity of the hosts to survive and reproduce; they have to release their symbiotic bacteria before intensive host immune responses occur. The goal of this study is to understand the immune responses between two species of nematodes, Heterorhabditis bacteriophora and Steinernema glaseri and their insect hosts, and the relationship to host specificity. The insect hosts we tested are Wax worm Galleria mellonella, Oriental beetle larvae Exomala orientalis, Japanese beetle larvae Popillia japonica, tobacco horn worm Manduca sexta, Northern masked chafers larvae Cyclocephala borealis, and house cricket Acheta domestica. We found that H. bacteriophora and S. glaseri infected wax worms and reproduced well. Both H. bacteriophora and S. glaseri killed most Japanese beetle larvae, Oriental beetle larvae, and tobacco horn worms. S. glaseri reproduced better than H. bacteriophora in these insect hosts. Interestingly, the S. glaseri NC strain has stronger pathogenicity compared to the S. glaseri FL strain in the same hosts. Northern masked chafers larvae and house cricket are resistant hosts to both nematodes. In injection assays, we found that in M. sexta, P. japonica and E. orientalis, high percentages of H. bacteriophora were melanized while high percentages of S. glaseri were moving freely. In M. sexta and P. japonica, higher percentages of S. glaseri FL were encapsulated compared to S. glaseri NC. In resistant host C. borealis, both H. bacteriophora and S. glaseri were melanized. Our results support the nematodes elicit immune responses in hosts that correlate with their infectivity. Using an in vitro assay, we also found that hemocytes from M. sexta recognized S. glaseri at a low percentage during the first hour post nematode introduction, and after 24 hours, H. bacteriophora escaped recognition of G. mellonella blood cells.

OTHER

Poster / Other. O-1

The Sleeping Beauty transformation system: A new approach for the study of tick cell microbe interactions
Timothy J. Kirk1, Roderick F. Feltsheim2, Gerald D. Baldridge3, Nicole Y. Burkhardt1, Michael J. Herron3 and Ulirike Munderloh4

1Department of Entomology, University of Minnesota, 1980 Folwell Avenue, St. Paul, MN 55108, USA

Transgenesis and paratransgenesis offer powerful approaches for analysis of molecular interactions between arthropod vectors, their microbial symbionts, and human pathogens. We addressed the need for transformation systems for the study of ticks and their associated microbes by exploring the ability of the Sleeping Beauty transformation system, a reconstructed Tcl/mariner related transposable element and a reconstructed transposase from teleost fish sequences (Ivics et al. 1997 Cell 91:501), to mediate transfection of ixodid tick cells. Cell line IS6E6 from the black legged tick, Ixodes scapularis, was stably transformed using marked Sleeping Beauty transposons in the presence of plasmids expressing transposase. Marker genes were either red fluorescent protein (DsRed2) or neomycin resistance genes. Transient expression of DsRed2 lasted 4 weeks and by 6 weeks approximately 90% of the cells lost expression of DsRed2. The remaining positive cells were stably transfected, and selectable using a neomycin analog, G418. Cloning and sequencing of the integration sites demonstrated that insertions of the DsRed2 gene within the cells’ genome occurred via the action of the Sleeping Beauty transposase. This system has potential for functional genetic analysis of interactions between ticks and microorganisms.

Poster / Nematodes. N-5

Infectivity of entomopathogenic nematodes and immune responses of their insect hosts
Xinyi Li1, Richard S. Cowles2, Elizabeth Cowles3, Randy Gaugler4 and Diana L. Cox-Foster4

1Department of Entomology, The Pennsylvania State University, University Park, PA 16802, 2Valley Laboratory, The Connecticut Agricultural Experiment Station, Windsor, CT 06095, 3Department of Biology, Eastern Connecticut State University, Willimantic, Connecticut 06226, 4Department of Entomology, The Rutgers University, New Brunswick, NJ 08901, USA

Entomopathogenic nematodes (EPNs) are ecologically and economically important. Two families of insect EPNs are good candidates of biological-control agents, but they do not significantly reduce pesticide application because of their poor host suitability. Infective juveniles (IJ) of EPNs penetrate insect hosts and release symbiotic bacteria that kill the insect hosts and serve as food resources for EPNs. Insect hosts defend against EPNs by a rapid cellular immune response resulting in encapsulation and melanization that kills EPNs. The nematodes have to overcome the innate immunity of the hosts to survive and reproduce; they have to release their symbiotic bacteria before intensive host immune responses occur. The goal of this study is to understand the immune responses between two species of nematodes, Heterorhabditis bacteriophora and Steinernema glaseri and their insect hosts, and the relationship to host specificity. The insect hosts we tested are Wax worm Galleria mellonella, Oriental beetle larvae Exomala orientalis, Japanese beetle larvae Popillia japonica, tobacco horn worm Manduca sexta, Northern masked chafers larvae Cyclocephala borealis, and house cricket Acheta domestica. We found that H. bacteriophora and S. glaseri infected wax worms and reproduced well. Both H. bacteriophora and S. glaseri killed most Japanese beetle larvae, Oriental beetle larvae, and tobacco horn worms. S. glaseri reproduced better than H. bacteriophora in these insect hosts. Interestingly, the S. glaseri NC strain has stronger pathogenicity compare to the S. glaseri FL strain in the same hosts. Northern masked chafers larvae and house cricket are resistant hosts to both nematodes. In injection assays, we found that in M. sexta, P. japonica and E. orientalis, high percentages of H. bacteriophora were melanized while high percentages of S. glaseri were moving freely. In M. sexta and P. japonica, higher percentages of S. glaseri FL were encapsulated compared to S. glaseri NC. In resistant host C. borealis, both H. bacteriophora and S. glaseri were melanized. Our results suggest the nematodes elicit immune responses in hosts that correlate with their infectivity. Using an in vitro assay, we also found that hemocytes from M. sexta recognized S. glaseri at a low percentage during the first hour post nematode introduction, and after 24 hours, H. bacteriophora escaped recognition of G. mellonella blood cells.

OTHER
that binds to the salivary gland and importantly, inhibits parasite invasion. By use of a combination of approaches including UV-crosslinking pull-down experiments, Western blotting of sporozoite proteins with an anti-SM1 antibody and protein identification using mass spectrometry, we have identified a putative salivary gland receptor (the surface protein suglin) and the interacting sporozoite surface protein (TRAP). We will present data that led to these findings and discuss its implications for future work.

Symposium, Wednesday, 5:00.

Relationships between the symbiont Sodalis glossinidius and the vectorial competence of tsetse flies

Anne Geiger, Gerard Cuny and Roger Frutos
UMR 17, IRD-CIRAD, CIRAD TA 207/G, Campus International de Baillarguet, 34398 Montpellier cedex 5, France.

Trypanosoma congolense, transmitted by Glossina or tse-tse flies, causes animal trypanosomiasis, or Nagana, in sub-Saharan Africa. Despite progress in the understanding of the pathogenesis, Nagana still generates dramatic economic damage. New strategies of control are investigated which require a better understanding of the transmission mechanisms of the parasite. Sodalis glossinidius, the secondary symbiont of Glossina is an enterobacteria suspected to play a role in the establishment of the parasite and in vectorial competence through specific tripartite interactions. We investigated the differential presence of S. glossinidius in T. congolense-infected and non infected midguts of Glossina palpalis gambiae and Glossina morsitans morsitans, respectively poor and major vectors of the parasite, and in probosces of flies displaying mature or immature infection. S. glossinidius was detected in midguts from both Glossina species and in all probosces from G. p. gambiae displaying mature or immature infection, but never in G. m. morsitans. S. glossinidius is probably not involved in T. congolense maturation, but could participate to the establishment process. Genetic diversity among S. glossinidius populations was investigated using AFLP markers. Isolates from each Glossina species are genetically distinct and group into separate clusters. This correlates with the differing vectorial abilities of Glossina.

Symposium, Wednesday, 5:30.

Functional genomics in the postgenomic era: What do we learn from the apicomplexan malaria parasite?

Liwang Cui
Department of Entomology, Penn. State University, USA

The genome sequencing projects of several important apicomplexan parasite species have contributed greatly to our understanding of the parasite metabolism, evolution and pathobiology. The sequencing of several malaria parasite species has revealed novel metabolic pathways, identified a novel organelle - the apicoplast, and determined molecules that are potential targets for drug and vaccine development. In the postgenome era, analytic tools such as microarrays and proteomics allow gene analysis to be performed on a genome-wide scale. The recently developed transfection technology for the malaria parasites has further enabled functional analysis of individual genes through targeted gene disruption. To understand the transcription regulation in the malaria parasites, we have undertaken efforts to study the effects of dynamic chromatin modifications in gene silencing and activation. We have begun to characterize enzymes and their complexes that covalently acetyle histones. This study may yield new information about the roles of these evolutionarily conserved enzymes in transcription regulation in this group of lower protozoan parasites. This may further establish a direct link between histone acetylation and parasite virulence, which is mostly determined by the monoallelic expression of surface variant proteins. Technologies and results obtained should be applicable to other apicomplexan parasites.

Symposium, Wednesday, 6:00.

Sand fly midgut receptors for Leishmania parasites: Targets for transmission-blocking vaccines

Shaden Kanhai1, Marcelo Ramalho-Ortigao1, Van M. Pham1, Sanjeev Kumar2, Phillip G. Lawyer3, Salvatore J. Turco2, Carolina Barillas-Mury1, David L. Sacks2, Jesus G. Valenzuela1

1Vector Molecular Biology Unit, Laboratory of Vector Biology, NIAID, National Institutes of Health, Bethesda, MD 20892, USA, 2Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD 20892, USA, 3Mosquito Immunity and Vector Competence Unit, Laboratory of Malaria and Vector Research, NIAID, NIH, MSC 8132, Bethesda, MD 20892-8132, USA

We have isolated from a P. papatasi sand fly midgut cDNA library a transcript coding for a galecin (PpGalec). The observed homology of PpGalec to galactose-binding proteins, together with previous studies indicating that poly-Gal (B1-3) side chains of Leishmania major lipophosphoglycan (LPG) are responsible for specific binding to P. papatasi midguts, suggested that PpGalec is the midgut receptor for L. major in this sand fly species. Expression of PpGalec in P. papatasi is upregulated in adult females and is restricted to midgut tissue; expression of PpGalec is restricted to P. papatasi and P. duboscqi sister species belonging to the subgenus Phlebotomus. The binding specificity of recombinant PpGalec is restricted to Leishmania promastigotes bearing poly-Gal (B1-3) side chains on their LPG. PpGalec is localized on the luminal surface of midgut cells. Interestingly, antibodies against PpGalec inhibited ex vivo midgut binding of L. major PG and parasites, additionally, PpGalec antibodies fed to P. papatasi severely impaired parasite development and survival in the insect midgut. This is the first molecular description of a parasite receptor in the midgut of its insect vector. In addition, these studies demonstrate the feasibility of using midgut receptors for parasite ligands as target antigens for transmission-blocking vaccines.
Granulovirus targets larvae before or during initial entry into fruit and stings and larval mortality was consistently high. Higher doses were safe to humans and natural enemies. Our objectives for the 2004 field season were to assess full-season virus programs adopting different application rates (1, 3, or 6 oz/acre) and spray intervals (7, 10, or 14 days) in an experimental orchard and to compare different rates of virus (1, 2, or 3 oz/acre) applied weekly to Guthion in a conventionally managed orchard heavily infested with CM. Virus applications did not reduce fruit damage by CM, but there were significantly fewer deep entries and surviving larvae among virus-treated plots compared with untreated plots. The dosage and application frequency of virus that provides protection provided by the navel end protect position in the soil. Biopesticides are known to be active in the soil and are considered difficult targets for eradication due to their cryptic, usually long-lived and exist in localised areas in the first stages of colonisation. These conditions favour pathogen based control which is necessary to intercept and eradicate potential pest species before they can become established. Soil inhabiting pests are a particular problem necessary to intercept and eradicate potential pest species before they can become established. Soil inhabiting pests are a particular problem for eradication due to their cryptic, protected position in the soil. Biopesticides are known to be active in the soil and are considered difficult targets for eradication due to their cryptic, usually long-lived and exist in localised areas in the first stages of colonisation. These conditions favour pathogen based control which has the potential to become an effective tool for eradication of exotic pests if virulent pathogens and appropriate tactics can be used.

Can pathogens be used for eradication of soil pests?
Trevor A. Jackson, Todd Kleinschafer and Richard J. Townsend
AgResearch, PO Box 60, Lincoln, New Zealand

Invasions by exotic pests are a negative side effect of greater international trade and tourism. If indigenous flora and fauna are to be protected in the more unique corners of the planet, it will be necessary to intercept and eradicate potential pest species before they can become established. Soil inhabiting pests are a particular problem for eradication due to their cryptic, protected position in the soil. Biopesticides are known to be active against these pests but often efficacy is limited in the field. We have tried a range of tactics to overcome these limitations for the elimination of pest scarabs from microplots. These include manipulation of dose rate, multiple pathogen application and formulation. High doses of pathogen when correctly timed and well distributed through the soil can produce high mortality in the target population. Alternatively where multiple pathogens are used, synergism can raise the level of infection in the target insects. Using microbial formulation to maintain a high level of infective propagules in the soil may also overcome the problem of finding the correct application time for short-lived microbes. Soil inhabiting insects are usually long-lived and exist in localised areas in the first stages of colonisation. These conditions favour pathogen based control which has the potential to become an effective tool for eradication of exotic pests if virulent pathogens and appropriate tactics can be used.

Efficacy of entomopathogenic nematodes, applied in an insect cadaver, as biological control agent against soil-dwelling stages of boilworm (Helicoverpa armigera Hübner).
Astrid Jankielsohn and Justin L. Hatting
ARC-Small Grain Institute, Private Bag X29, Bethlehem, 9700, South Africa

The efficacy of using an insect cadaver to apply entomopathogenic nematodes (EPNs) was tested in greenhouse studies against soil-dwelling stages of Helicoverpa armigera. The EPN strains screened included two Heterorhabditis, SGI 22 and SGI 173, and one Steinernema, SGI 148. The host used for application of nematodes was highly susceptible to all three strains with SGI 148 and SGI 173 both causing 100% and SGI 22 causing 69% mortality. Cadavers
infected with SGI 173 produced the highest mean number of IJs of 119025, emerging over 20 days. SGI 148 and SGI 22 produced a mean of 72258 IJs emerging over 29 days and 44226, over 22 days, respectively. Preliminary observations showed that soil-dwelling life stages of Heliothis armigera were susceptible to all EPN strains tested. Against the pupal stage, the most virulent strain was SGI 173, causing 88% mortality, while SGI 22 and SGI 146 caused 68.75% and 83% mortality over six days, respectively. Observations following the six-day period will quantify survival and infective capacity of IJs in the soil without a host. This will be accomplished by periodic introductions of healthy larvae onto the plants allowing for natural migration into the soil. Results will be discussed.

Contributed paper. Wednesday, 6:00. 155

**Yanhua Jia** 1,2, Jie Zhang1 and Guoxun Li3

Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany

In the year 2003, field trials against *Thrips tabaci*, were conducted at four different sites with commercially available products based on insect pathogenic fungi and nematodes. The trials were performed according to the EPPO guideline PP 1/85(3). As a chemical standard Perfekthion® was used in onion. In leek, Spruzit® and Neudosan® were applied alternately. The biocontrol products used were Mycotal®, PreFeRal®, Naturalis L®, Nemaplus® and Nemagreen®. They were applied either alone or in a mixture using common spray equipment. The treatments started mid June and were repeated up to six times in weekly intervals. At all four sites, *T. tabaci* was recorded only in medium numbers (30 per plant) throughout the summer. In two trials, no differences were detected between the treatments and the control. In the third trial (onion), a significant reduction was recorded for the treatment 'PreFeRal+Nemaplus', both in the number of thrips/plant and the frequency of infestation (38% compared to 93% in the control). Also in the fourth trial (leek), the number of thrips/plant was lowest for the treatments 'fungi+nematode'. In this trial, yield was measured additionally, and the weight/plant was 20% higher for the treatments 'Nemaplus' and 'Mycotal+Nemaplus' compared to the control (425 and 412 g/plant compared to 345 g respectively).

**STU** Contributed paper. Wednesday, 6:15. 154

**Construction of the rfp gene marker system to monitor insecticidal and anti-fungi engineered bacterium of *Pseudomonas fluorescens* Biop8**

Yanhua Jia1, Jie Zhang1 and Guoxun Li2

1 State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100094, P.R China, 2 Agricultural University of HeBei, Baoding 071001, P.R China, 3 Agricultural College of LaiYang, LaiYang 265200, P.R China

*Pseudomonas fluorescens* is an important biocontrol bacterium for agriculture. The engineered bacterium, Biop8 that *P. fluorescens* P303 was obtained with *crynAb, cry1Ac* and *cry2Aa* from *Bacillus thuringiensis* in two kinds of different brushes plasmid vectors, is toxic active against insects and suppresses plant pathogenic fungi. In this study, in order to monitor the exist situation of Biop8 in environment, the *rfp* gene was introduced into Biop8 via Mini-Tn5, yielding the chromosome marker conjugant 8’Biop8. The stability of the marked strains was outstanding. The beneficial anti-fungal characteristic of the host strain was not affected by expression of the foreign gene *rfp*. The test of marked strains in the soil indicated that the marked strains were safe for the environment and didn’t affect the indigenous bacteria in the soil. As a marker gene for *P. fluorescens* the *rfp* gene express the *gfp* gene in a number of ways. A simple and sensitive tool, confocal scanning laser microscope (CSLM), should be considered for detection of the marked microorganisms.

**STU** Contributed paper. Wednesday, 5:00. 157

The effect of tannic acid on gypsy moth performance and susceptibility to the nuclear polyhedrosis virus

V. Martemyanov, Z. Markina, S. Romancev, Stavislav A. Bahvalov

Laboratory of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia

The effect of tannic acid, which is one of the chemicals involved in the immune response of woody plants caused by defoliation, on the
The performance of gypsy moth (Lyma
tria dispar L.) and its susceptibility to nuclear polyhedrosis virus (LdNPV) was studied. The following parameters were estimated: larvae weight, mortality rate, activities of detoxification enzymes (glutathione-S-transferase, and esterase) and antioxidant enzymes (superoxide-dismutase, catalase), and thiol content in the tissue of insect midgut. Tannic acid was found to increase susceptibility of gypsy moth larvae to LdNPV by feeding on artificial diet. Larval mass was increased too under tannic acid and virus treatment in comparison with control. But sensitivity of larvae fed on natural diet with tannic acid to LdNPV was decreased. It was also found that the concentration of oxidized thiols in midgut of larvae fed on as natural as artificial diet was increased under effect of tannic acid and virus. This result testifies to increase of oxidizing process in insect midgut. It was shown that the increased concentration of tannic acid benefited insects in the “host-plant-herbivore-virus” interaction, though most likely the defoliation-induced tannic acid in vivo may act otherwise.

**STU Contributed paper. Wednesday, 5:15. 158**

**Stimulation of cell motility by a viral fibroblast growth factor homolog: Proposal for a role in viral pathogenesis**

Chanutcho Detvisitsakun, Marcello F. Berretta, Christopher Leihy, and A. Lorenza Passarelli

Division of Biology, Molecular, Cellular, and Developmental Biology Program, Kansas State University, Manhattan, KS 66506–4901, USA

The Autographa californica nucleopolyhedrovirus (AcMNPV) encodes a gene (open reading frame 32) with homology to vertebrate and invertebrate fibroblast growth factors (fgf), key regulators of developmental processes affecting the growth, differentiation, and migration of many cell types. We studied the temporal regulation of the AcMNPV fgf, vfgf, by Northern (RNA) blot hybridization; vfgf was transcribed as a 0.6-kb mRNA at early times but as part of a 1.4-kb bicistronic mRNA at late times. The product of vfgf, VFGF, exhibited a number of characteristics that have also been demonstrated for other FGF homologs. VFGF had strong affinity to heparin, a property important for FGF signaling via an FGF receptor. VFGF was secreted into the extracellular fluid when expressed in insect cells, suggesting that it acts as an extracellular ligand. Finally, VFGF was able to stimulate migration of several different types of insect cells. We discuss how this activity may be important for its function during virus infection.

Contributed paper. Wednesday, 5:30. 159

**Pathology of NeaNPV-infection in balsam fir sawfly, Neodiprion abietis larvae.**

Beatrice Whittome1, Benoit Morn2, Christopher Lucarotti2, Dan Quiring3, and David Levin1

1University of Victoria, Victoria, BC, Canada, 2Natural Resources Canada, Canadian Forestry Service, Fredericton NB, Canada, 3Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, NB, Canada

Unlike NPVs of lepidopteran hosts, little is known about the pathology of hymenopteran-derived viruses, or even about the morphology of the tissues they infect. We are characterizing the pathology of Neodiprion abietis nucleopolyhedrovirus (NeaNBPV) in virus-infected larvae of the Balsam fir sawfly. Second and third instar larvae of N. abietis were infected with 1000 NeaNBPV polyhedra per os and were then perfused and dissected under Karnovsky’s fixative at 72, 48, 24, and 12 hours post inoculation, along with uninfected controls. The intact guts were dehydrated with ethanol and embedded into LR gold. Semi-thin (500 nm) and ultra thin (70nm) sections were obtained by sampling the gut cross-sectionally every 100 μm throughout the entire gut length. Semi-thin sections were stained with Richardson’s stain and microphotographs were obtained. At 72 hours post inoculation, the anterior third of the midgut showed visible signs of infection, including hypertrophied nuclei and polyhedra. The degenerative cells, found only at the foregut-midgut junction, were also infected and were sloughing off into the gut lumen. Early time points in the pathological process were less distinct in regards to their cytopathic effects and will be studied by immunofluorescent LM and immunogold TEM.

Contributed paper. Wednesday, 5:45. 160

**Characterization of Helicoverpa armigera nucleopolyhedrovirus ORF2**

Yanchao Nie1,2, Qian Wang1,3, Changyong Liang1,3, Zehua Yu1 and Xinwen Chen1

1State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Virology, Wuhan 430071, 2Institution of Entomology, Central China Normal University, Wuhan 430070, 3Graduate School of the Chinese Academy of Sciences, Beijing, 100039, People’s Republic of China

It was shown that nucleocapsids of baculoviruses are capable of nucleating actin polymerization in vitro. Sequence analysis shows that the open reading frame 2 (Ha2) of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus (HaSNPV) contains common motifs of WASP or WASP-like protein such as RickA and ActA, suggested that baculovirus might use the WASP-Arp2/3 pathway to achieve nucleocyttoplasmic transport. Ha2 is 1242 bp long and encodes a protein with predicted 45.9 kDa. RT-PCR results show that Ha2 transcripts were detected from 16 to 72 h post infection (hpi). Polyclonal rabbit antiserum was raised to a GST-HA2 fusion protein; Western blot analysis detected three protein bands in size of 50, 46 and 35 kDa in infected-HzAM1 cells from 24 to 72 hpi. However, only the 50 kDa form was detected in BV and ODV. EGFP-Tag analysis showed that HA2 was localized primarily in the nucleus of HzAM1 cells. With the labeling of actin by Rhodamine-Phalloidin, HA2 was found to colocalize with actin.

Co-opting actin and the Arp2/3 complex for baculovirus progeny production

Erin Goley1, Taro Okhawa1, Matthew Welch1, and Loy Volkman1,2

1Department of Molecular and Cell Biology, and 2Department of Plant and Microbial Biology, University of California, Berkeley CA 94720-3102, USA

AcMNPV and other baculoviruses with larval lepidopteran hosts depend on host actin for progeny virus production. Actin recruitment to the nucleus is dependent on early gene expression, and actin polymerization within the nucleus is dependent upon late gene expression. Arp2/3 is a 7-member complex of host proteins that choreographs actin nucleation and organization at the leading edge of migrating animal cells. When activated by the Wiskott-Aldrich Syndrome protein (WASP), the Arp2/3 complex nucleates actin polymerization. Interestingly, the Arp2/3 complex is recruited to the nucleus during AcMNPV infection, and the minor capsid protein of AcMNPV, p78/83, has the capability of substituting for WASP in Arp2/3-dependent actin-polymerization reactions in vitro. Mutant variants of p78/83 show a range of abilities to activate the Arp2/3 complex, and these abilities directly correlate with progeny virus production levels when substituted into AcMNPV genomes and used to infect cells. Mutants that cannot activate Arp2/3 cannot produce virus. This represents the first known role for Arp2/3 in the nucleus.

Analysis of the ability of exon 0 homologues from heterologous baculoviruses to complement an AcMNPV exon

Xiaojiang Dai1,2, Basim M. Ari1, Peter J. Krell1, and David A. Theilmann1

1Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C. V0H 1Z0, Canada, 2Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada, 3Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste Marie, Ontario P6A 2E5, Canada

Exon 0 (orf141) of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is required for efficient production
of budded virus in the AcMNPV life cycle. This gene is highly conserved throughout the baculovirus Group I and Group II NPVs but it is not known if this important gene is virus or host specific. To test virus specificity an expression examination was conducted by conducting the first microsporidian EST sequencing. This survey showed that many transcripts encode fragments of or complete copies of several genes. These multi-gene transcripts are not polyctronic: instead promoters and terminators were squeezed from shrinking intergenic regions into adjacent genes. To determine if this was caused by compaction, we sequenced ESTs from even more highly compacted genomes (nucleomorphs) of endosymbiotic green and red algae. These possess multi-gene transcripts at even higher frequencies. Altogether, compaction may have subtle but important effects on genome functions, and also points to potential challenges in studying expression in these systems (e.g., using arrays), since the actual target of expression can be difficult to discern.

Microsporidian parasites of crustacea, specificity, sex and populations
Judith E. Smith, Johanna G. M. Slothouber Galbreath, Yang Qui, James J. Becnel and Alison M. Dunn

1School of Biology, University of Leeds, Clarendon Way, Leeds, LS2 9JT, UK, 2School of Biological Sciences, University of Aberdeen, Zoology Building, Aberdeen AB24 2TZ, UK, USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, P.O. Box 14565, Gainesville, FL 32604, USA

Methods for analyzing data from bioassays with insect pathogens
James E. Throne

Methods for analyzing data from bioassays with insect pathogens
James E. Throne

USDA-ARS Grain Marketing and Production Research Center, Manhattan, KS 66502, USA

Methods for analyzing data from dose-response and time-response bioassays with insect pathogens will be presented. Topics will include dose (or time) selection, number of replicates and number of individuals to test per replicate (and what to do with replicates in analyses), computer programs available for analyzing data, how to compare responses to different treatments, and what to report in publications. Examples and interpretation of complete analyses of dose-response and time-response bioassay data will be discussed. The talk is part of a workshop, so the format will encourage questions about these topics and related topics during the talk and discussion among workshop participants.

Epizootiology of the microsporidium Thelohania solenopsae in the red imported fire ant, with emphasis on social form of the host
James R. Fuxa, Maynard L. Milks, Yuliya Y. Sokolova, and Arthur R. Richter

Department of Entomology, Louisiana State University AgCenter, Baton Rouge, LA 70803, USA

Epizootiology of the microsporidium Thelohania solenopsae was investigated in red imported fire ants, Solenopsis invicta. The microsporidium was detected at 16% of 165 sites and in 10% of 1309 colonies surveyed throughout Louisiana. Its random distribution was not affected by type of habitat. It infected 2.4% of monogyne (single-queen) and 53.3% of polymege (multiple-queen) colonies. Its occurrence was positively correlated with number of colonies and with four soil parameters. Microsporidium-infected colonies in the survey were less likely to have brood than healthy colonies. A natural T. solenopsae epizootic was monitored in a mixed monogyne/polygyne ant population. The 89-100%-infected polymege ants disappeared, possibly because they were at a competitive disadvantage to 15-26%-infected monogyne ants. The monogyne form did not sustain the pathogen after polymege ants disappeared. Long-term epizootics developed when the microsporidium was released in two predominantly polymege populations but not at two monogyne sites. In mixed host populations, prevalence peaked at
The genus *Brachiola* and human skeletal muscle infection caused by the mosquito microsporidium, *B. algerae*

Ann Cali1, L.M. Weiss2, and Peter M. Takyvorin3

1Department of Biological Sciences, Rutgers University, Newark, NJ 07006, USA, 2Departments of Pathology and Medicine, Albert Einstein College of Medicine, NY 10461, USA

The genus *Brachiola* was established and placed within the family Nosematidae, to accommodate organisms that share the features of that family but also possess some unique features including several variations of the plasmodialma, adaptation to higher host body temperatures, and specifically, with the features of the type species, *vesicularum*, the ability to extend its protoplasm into long branches named protoplasmic extensions (often longer than the parasite cell itself) while maintaining the plasmemmal variations demonstrated on the main body of the parasite cells. This parasite, *B. vesicularum*, was described from human skeletal muscle infection in an individual who was immune compromised. Some of the features of this parasite are shared with the parasite formerly known as *Nosema algerae*, however, after much ultrastructural examination of it, we concluded that they were closely related but morphologically differing in the presence or absence of the protoplasmic extensions, which have not been demonstrated on any other microsporidium. An evaluation of the extensive literature on *N. algerae*, revealed the consensus that molecularly it was considered an “out group” and should probably be in another genus. Thus, the genus *Brachiola* was established to accommodate the new parasite, *B. vesicularum*, and the organism, *N. algerae*, was transferred into it, becoming *B. algerae*. In other studies, we and others presented the ability of *B. algerae* to tolerate higher temperatures than previously reported and in 2004, it was demonstrated as the causative agent in a case of severe myositis of a woman with rheumatoid arthritis who was being treated with immunosuppressive drugs for the treatment of that disease. After extensive ultrastructural examination, it was concluded that in an environmental situation virtually identical to that in which *B. vesicularum* had formed the protoplasmic extensions, *B. algerae* did not develop them, thus demonstrating a significant morphological difference between the two species. This infection was molecularly proven to be *B. algerae* with a greater than 99% match using the *B. algerae* primers and sequence data from genbank. Thus, this mosquito-infecting microsporidium did not cause myositis and ultimately lead to the death of a 57-year-old woman.

**VIRUSES 4**

**CONTRIBUTED PAPERS. Thursday, 8:00-10:00**

**Establishment of a natural phylogeny of Baculoviruses**

Rüdiger Hauschild1, Martin Lange1, Olaf Bininda-Emonds2, Johannes A. Jehle1

1Labor für Biotechnologischen Pflanzenschutz, Dienstleistungszenrum Ländlicher Raum Rheinpfalz, Breiteng 71, 67435 Neustadt/Wst, Germany, 2Lehrstuhl für Tierzuch, Technische Universität München, Hochfeldweg 1, 85354 Freising-Weihenstephan, Germany

More than 600 Baculovirus isolates from Lepidoptera, Hymenoptera, and Diptera are known to date. Their genome consists of 80-180 kbp double-stranded DNA. The family Baculoviridae contains diverse members, and classification is based on virus morphology and host association. However, this classification does not represent phylogeny of this virus group. For example, Lepidopteran specific members of the genus Nucleopolyhedrovirus are more closely related to the genus Granulovirus than to other Nucleopolyhedrovirus infecting Dipteran or Hymenopteran hosts. In addition, virus classification by the host leads to misunderstandings or ambiguous species names if different virus infect the same host or if the same virus infects different insect hosts. In order to determine and to classify virus isolates on a molecular level, degenerate primers were developed and used to amplify regions of the genes encoding lef-8, lef-9, and polyhedrin/granulin. These partial gene sequences permit establishment of phylogenetic relationships between different virus isolates and species classification. Ambiguous species names could be detected. To establish a natural phylogeny, sequences of 62 conserved genes from all completely sequenced baculovirus genomes together with the sequence information of more than 100 data sets for lef-8, lef-9, and polyhedrin/granulin were compared using a supertree approach. A Maximum-Likelihood analysis with bootstrap support is done for each gene according to the most appropriate model of evolution. These trees are then used to perform a weighted supertree analysis to determine the phylogenetic tree for the family Baculoviridae. In the same time, evolution of Lepidopteran hosts is assessed using amplification and analysis of partial sequences of the gene encoding sub-unit I of cytochrome-oxidase (COX I). This information together with the baculovirus phylogeny allows an unambiguous identification of baculovirus-host associations and provides insight into the evolutionary relationships between Baculovirus and their Lepidopteran hosts.

**STU** Contributed paper. Thursday, 8:15. **169**

Whole genome sequence analysis of a Polish isolate of *Agrotis segetum* nucleopolyhedrovirus

Agata K. Jakubowska1, René M. Klein Lankhorst2, Jadwiga Ziemnicka1, Just M. Vlak2 and Monique M. van Oers2

1Department of Biological Control and Quarantine, Institute of Plant Protection, Mieczurza 20, Poznan, 60-318, Poland, 2Laboratory of Virology, Wageningen University, Binnenhaven 11, Wageningen, 6709 PD, The Netherlands, 3Gren明朝, Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

The tump moth, *Agrotis segetum*, is an important pest in many crops in Europe, Asia and Africa. Both nucleopolyhedrovirus (NPV) and granulovirus (GV) species were isolated from this insect with biocontrol potential in the field. The genome of *A. segetum* GV (AgseGV) has been completely sequenced (Ai et al., 2004 NC 005839). Two different *A. segetum* NPVs have been isolated in the United Kingdom (AgseNPV-UK) and in Poland (AgseNPV-P). Phylogenetic analysis based on three conserved baculovirus genes, polyhedrin, late expression factor 8 (lef-8) and the per os activity factor 2 (pof-2), revealed that both isolates show an evolutionary relationship with group II NPVs and probably represent different virus species (Jakubowska et al., 2005, submitted). The genome sequence of AgseNPV-P is 147,543 bp long and has an GC-content of 45.7%. In addition to 62 genes common for lepidopteran baculoviruses, including the 29 core genes, a few ORFs unique for AgseNPV-P were found. In gene content and gene order, AgseNPV-P is strikingly similar to *Spodoptera exigua* (Se) MNPV. The major difference is the presence of three enhancin genes in AgseNPV-P. Few ORFs with high homology to granulovirus genes are present in AgseNPV-P, suggesting gene transfer between NPVs and GV's infecting the same host.

**166** Contributed paper. Thursday, 8:00. **170**

Genome sequence and organization of the *Neodiprion abietis* nucleopolyhedrovirus.

Simon Duffy1, Benoit Morin2, Christopher Lucarotti2, and David Levin3

1University of Victoria, Victoria, BC, Canada, 2Natural Resources Canada, Canadian Forestry Service, Fredricton NB, Canada

The *Neodiprion abietis* nucleopolyhedrovirus (NabbNPV) is a naturally occurring virus (Family: Baculoviridae) pathogen of the balsam fir sawfly (*Neodiprion abietis*). Little is known about the genomics of sawfly-infecting baculoviruses but genome sequence analyses of the *Neodiprion lecontei* and *Neodiprion sertifer* NPVs
have been completed recently. The genome sequence of NeabNPV contributes to the growing knowledge base of sawfly-infecting NPV pathologies. Like the N. lecontei and N. sertifer NPVs (NeseNPV and NsCPV, respectively), NeabNPV has a relatively small genome (85Tkb) with a high A-T residue content (67%). While NeabNPV appears to be most closely related to the N. lecontei NPV in gene content (94%), comparative analyses of the three sawfly NPV genomes indicates that they are distinct from one another and have genome specific arrangements. While approximately two thirds NeabNPV genome displays high sequence parity (a high level of sequence similarity, gene content, and gene order) with the NeseNPV and NeseNPV, the remaining region of NeabNPV (approximately position 0-30 kb) is highly variable in gene content. The significance of the conserved and variable regions with respect to pathology of NeabNPV will be discussed.

Contributed paper. Thursday, 8:45.

Morphological, molecular, and genomic characterization of two mosquito Cypoviruses
Terry B. Green1, Alexandra Sharpio1, Susan White1, Shujing Rao2, Peter Mertens3, Gerry Carner4, and James B. Becnel1

1USDA/ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600-1700 S.W. 23rd Drive, Gainesville, FL 32608, USA, 2Burgh Hid Laboratory, Institute for Animal Health, Ash Road Pitbright, Woking, Surrey, GU24 0ET, UK, 3Clemson University, 114 Long Hall, Clemson, SC 29634-0315, USA

The morphological, genomic, and molecular characteristics of two cypoviruses (cytoplastic polyhedrosis virus, CPV) from the mosquitoes Colex restuans (CrCPV) and Uranotaenia sapphirina (UsCPV) are examined in this report. CrCPV is characterized by large, irregularly shaped inclusion bodies that are multiply embedded. This differs from UsCPV, which typically has a single virion per inclusion body and a regular cuboidal or spherical shaped inclusion body. The transmission rate for both Cypoviruses was enhanced by the presence of magnesium ions but was inhibited by calcium ions. Transmission studies have also shown that each of these Cypoviruses infect multiple mosquito species. CrCPV and UsCPV are the only two Cypoviruses from mosquitoes that have been confirmed by using molecular analysis. Both Cypoviruses have a 10 segmented genome that is quite different from the lepidopteran cypoviruses TrCPV-15 (Trichoplusia ni) and BmCPV-1 (Bombyx mori). In addition, nucleotide and amino acid analysis of segment 10 (polyhedrin) suggest that both cypoviruses are closely related to one another but unrelated to the sixteen remaining CPVs from lepidopteran hosts.

Contributed paper. Thursday, 9:00.

Integration of an ichnovirus genome segment in the genomic DNA of lepidopteran cells
Daniel Doucet1, Anic Levassure1, Catherine Béliveau1, Don Stoltz2 and Michel Cusson1

1Laurentian Forestry Centre, NRCan-CFS, 1055 du PEPS, Sainte-Foy, QC, G1V 4C7, Canada, 2Department of Entomology, University of Kentucky, Lexington, Kentucky, 40504-0091, USA

At the time of parasitization, the ichneumonid Trichoplusia ni demonstrated that it injects a polydnavirus that assists the wasp egg and larva in infecting the ecdysteroid hormone producing larvae and facilitates the insemination process of the wasp. At the time of parasitization, the ichneumonid Trichoplusia ni demonstrated that it injects a polydnavirus that assists the wasp egg and larva in infecting the ecdysteroid hormone producing larvae and facilitates the insemination process of the wasp. In vitro tests aimed at characterizing this T. rostrale ichnovirus (TrIV) demonstrated that it can infect cells of the Cf-124T cell line, and can express several viral genes. Remarkably, the gene TrFRep1, found on TrIV genome segment F, shows stable expression for at least 100 days after inoculation, compared to a few days for the other viral transcripts genes. Remarkably, the gene TrFRep1, found on TrIV genome segment F, shows stable expression for at least 100 days after inoculation, compared to a few days for the other viral transcripts.

The cloning and sequencing of segment F/Cf-124T genomic DNA junction sites, from a lambda genomic library, confirmed that the segment does integrate. Further experiments are under way to determine whether the phenomenon also occurs in vivo.

Contributed paper. Thursday, 9:15.

Comparison of genome organization and encoded proteins in campoplegine and banchine ichnoviruses
Renée Lapointe1, Bruce A. Webb2, Kojihiro Tanaka3, Walter Barney4, Don Stoltz2 and Michel Cusson1

1Laurentian Forestry Centre, NRCan-CFS, 1055 du PEPS, Sainte-Foy, QC, G1V 4C7, Canada, 2Department of Entomology, University of Kentucky, Lexington, Kentucky, 40504-0091, USA

Natural populations of the spruce budworm, Choristoneura fumiferana, are regulated by various natural enemies, including the ichneumonoid wasps Transosma rostrale (subfamily Campodeaglinateae) and Glypta fumiferana (subfamily Banchinae). These endoparasitoids oviposit at different times of their host’s life cycle: C. fumiferana females lay their eggs in pre-diapause 2nd instars, while T. rostrale wasps parasitize post-diapause instars. At oviposition, both parasitoids inject a polydnavirus (PDV) that is believed to be required for successful parasitization. Sequences obtained to date for genomes of PDVs isolated from ichnoviruses (Ichnovirus; IV) and baconid (Bracovirus; BV) wasps indicate that the two groups differ with respect to gene content and families. Here, we show that among IVs, there is intergeneric parasitoid specificity between T. rostrale and the banchine G. fumiferana (GfIV). The latter has a virion morphology distinct from that of campoplegine IVs, and its genome segments are both smaller and more abundant. In addition, sequence analysis indicates that while TrIV contains genes similar to those found in other IVs, the genes identified in GfIV are more similar to those of BVs. These singularities may reflect differences in phylogenetic lineages and/or in selection pressures experienced by the two wasps.

Contributed paper. Thursday, 9:30.

Display of a foreign protein using recombinant baculovirus occlusion bodies: A novel vaccination tool
Rebecca Wilson1, YeonHo Je1, Laurence Bugeon1, Ursula Straschil1, David R. O’Reilly1 and Julie A. Olszewski1,2

1Department of Biological Sciences, Imperial College London, SW7 2AZ, UK, 2Department of Genetics, Shippensburg University, Shippensburg PA, 17257, USA

Using an approach we developed to express foreign proteins within baculovirus occlusion bodies (OBs), we expressed the influenza A nucleoprotein (NP) as a fusion between two copies of the polyhedrin protein at the polyhedrin locus of AcMNPV. Reconstituent OBs with NP fusion protein were isolated and used to vaccinate mice. His-tagged soluble NP protein (His-NP), produced with conventional baculovirus expression technology, was used as a comparison for effectiveness of vaccination. The production of Ig antibodies, ratio of IgG2a to IgG1 antibodies produced, and lymphoproliferative response to NP challenge for excised lymph node cultures were measured and compared. His-NP protein (with adjuvant) gave a 10 fold better Ig titre than vaccination with NP-recombinant OBs. However, in lymphoproliferative assays, the NP-recombinant OBs gave a significantly higher stimulation index upon re-exposure to NP antigen than His-NP protein (with adjuvant). Additionally, vaccination studies showed that incorporation of foreign protein within OBs was more antigenic than simply co-injecting His-NP with wild-type OBs. Therefore, the ease of producing and isolating recombinant OBs which express good quantities of foreign protein, the stable nature of OBs, and the immune system stimulation by recombinant OBs all suggest that this approach could be a useful tool for vaccination of vertebrates.
Contributed paper. Thursday, 9:45. 175

**flashBAC: A baculovirus expression system for automated, high throughput production of proteins**

Linda King1, Kevin Richardson1, Richard Hitchman1, Helen Irving1, Susan Mann1, Evi Staat2 and Robert Possee2

1School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, OX3 0BP, 2NERC CEH Oxford, Mansfield Road, Oxford OX1 3QS, UK

The baculovirus expression system is traditionally viewed as one requiring a significant number of labour-intensive steps to produce recombinant proteins, particularly when producing multiple viruses simultaneously. We describe a new expression system, flashBAC that enables the production of multiple recombinant baculovirus expression vectors using automated, robotic systems. In this way we have been able to make 48 recombinant viruses per day using a relatively simple liquid handling robot. The system utilises a traditional plasmid vector to transfer the gene to be expressed into a modified AcMNPV genome; maintained as a low-copy number plasmid in bacteria. Following transfection of insect cells, recombinant occurs to produce initial stocks of recombinant virus. Because no selection step is required to separate recombinant from parental virus, a single researcher can handle the production of 48 recombinant viruses simultaneously, thus making this baculovirus expression system attractive to those with high throughput requirements. Small stocks of recombinant protein, sufficient for many screening purposes, have also been produced using the same automated, 24 well plate technology.

**CONTRIBUTED PAPERS. Thursday, 8:00-10:00**

**FUNGI 3**

Contributed paper. Thursday, 8:00. 176

**Field trials of Beauveria bassiana GHA for control of the emerald ash borer**

Houping Liu1 and Leah S. Bange1,2

1Department of Entomology, Michigan State University, E. Lansing, MI 48824, 2USDA Forest Service, North Central Research Station, E. Lansing, MI 48823, USA

The emerald ash borer, Agrilus planipennis, a buprestid native to Asia, was identified as the cause of ash (Fraxinus spp.) mortality throughout southeastern Michigan and southern Ontario in 2002. Infestations were later found in Ohio, Indiana, Maryland, Virginia due transport of infested nursery stock, firewood, and timber or natural spread. Regulatory agencies are attempting eradication of emerald ash borer through detection and removal of infested ash trees; this approach is both expensive and inadequate, as methods for detecting infested trees are lacking, and the infestation is far larger than predicted. We are working on the development of methods to manage emerald ash borer using microbial and biological control. After comparative bioassay of various fungi isolated from woodboring beetles against emerald ash borer, we focused our efforts on the use of Beauveria bassiana GHA against adult beetles due to its 1) virulence and 2) availability as a registered biopesticide. We will present the results of field trials conducted with B. bassiana GHA against emerald ash borer in 2003 and 2004.

**STU**

Contributed paper. Thursday, 8:15. 177

**A proactive approach to the use of fungal biopesticides to manage sucking insects in pulse crops in Australia**

Kristen Knight1,2, Caroline Hauxwell1, David Holdom1, Gordon Simpson1

1 DPI&F Biopesticides Unit, 80 Meiers Road, Indooroopilly 4068, Australia, 2School of Integrative Biology, University of Queensland, St Lucia 4068, Australia, 3Delivery, DPI&F, Tor St, Toowoomba, Queensland, 4350 Australia

Mirids (Cercopidae spp.) are the major pest to occur at the budding/flowering stage of mungbeans in Australia. Currently, mirids are controlled using “hard” chemical insecticides that also kill most beneficial insects, causing a dramatic increase of other pests, especially Helicoverpa spp. DPI&F are developing strategies for the use of fungal entomopathogens against mirids and green vegetable bug (Nezara viridula). The population dynamics of mirids in mungbeans was not clearly understood so season-long sampling was undertaken over three years in pulse crops and a model developed. This model can be used to predict points in the population development when a biopesticide could be applied proactively. The low impact on beneficial insects by Metarhizium anisopliae suggested an opportunity to develop proactive management strategies to reduce pest pressure before reaching threshold. A preliminary trial in mungbeans was undertaken in 2005. An early single application of an M. anisopliae isolate was as effective as the corresponding treatment of dimethoate in controlling mirids. The same M. anisopliae treatment did not “flare” Helicoverpa spp. or impact on any of the natural enemy species present. Future trials will compare the use of the model in proactive management with reactive application once the pest has reached threshold.

Contributed paper. Thursday, 8:30. 178

**Evaluation of some hyphomycetous fungi for the control of glassy-winged sharpshooter, Homalodisca coagulata (Homoptera: Cicadellidae)**

Surendra K. Dar1, Michael R. McGuire1 and Harry K. Kaya1

1Shafter Research and Extension Center, Shafter, CA 93211, 1USDA-ARS, Shafter, CA 93211, 2Department of Nematology, University of California, Davis, CA 95616, USA

Various assays were conducted evaluating Beauveria bassiana, Metarhizium anisopliae and Hirsutella spp. for the control of glassy-winged sharpshooter (GWSS), Homalodisca coagulata. Fungi were collected from infected GWSS or their habitats in Florida, Mississippi, Texas and California. Commercial isolates of B. bassiana and M. anisopliae and some isolates of the former from different hosts were also compared in various assays that evaluated different concentrations, inoculation methods and optimal incubation conditions. Differences were found among the isolates in their virulence to GWSS.

Contributed paper. Thursday, 8:45. 179

**Field testing of selected Beauveria bassiana isolates against Lygus hesperus in California**

Michael R. McGuire1 and Jarrod E. Leland2

1USDA-ARS, 17053 North Shafter Ave., Shafter, CA 93263, USA, 2USDA-ARS, Stoneville, MS 38776, USA

Naturally occurring isolates of Beauveria bassiana were isolated from Lygus spp. in California and Mississippi, characterized in the laboratory and field tested for activity and pest control potential against L. hesperus, the Western tarnished plant bug. Field treatments included: untreated control, GHA, a Mississippi isolate, a California isolate, and the chemical pesticide Warrior T. Applications were made in June and September 2005 (to a different set of plots) at a fungal rate of 1013 conidia/acre in June and 1014 conidia/ha in September. Population estimates and prevalence of infection were determined at -1, 3, 7, 10 and 14 days after application. All three B. bassiana treatments caused a high level of infection; >80% after the June application and >70% after the September application. However, populations did not significantly change immediately, except for the Warrior application in September. Within 10 days after application, populations of L. hesperus in all treated plots were significantly less than in control plots. No significant differences were observed among B. bassiana treatments. These results suggest B. bassiana may play some role in management of L. hesperus but further work will define the scope of this role.
Selection and field evaluation of Beauveria bassiana isolates for control of tarnished plant bug, Lygus lineolaris
Jarred E. Leland 1, Michael R. McGuire 1, and Jeff Gore 1
1USDA-ARS, SIMRU, Stoneville, MS, 38776, 2USDA-ARS, SREC, Shafter, CA, 93263, USA

Collections of Beauveria bassiana isolates from Lygus spp. populations in the Mississippi Delta and San Joaquin Valley of California were evaluated for characteristics relevant to field efficacy and mycoindirective development. Evaluation criteria included: pathogenicity to L. lineolaris and L. hesperus, impact on beneficial insects, spore production, mycoxin production, survival under solar radiation, and temperature tolerance. Based on these criteria one isolate from each collection was selected for field trials and comparison to the commercial B. bassiana isolate (GHA). Prevalence of infection and population change following application were determined for L. lineolaris and beneficial insect predators in wild host plants and cotton. Prevelance of infection in caged L. lineolaris adults were used to evaluate the persistence of B. bassiana conidia on plant surfaces. Results of field test conducted in 2004 and 2005 against L. lineolaris populations wild host plants and cotton will be presented.

Fungal BCAs: Potential control agents to control subterranean pests
Hermann Strasser 1, Barbara Pernfuss 1 and Roberto Kron Morelli 2
1Institute of Microbiology, Leopold Franzens University Innsbruck, 6020 Innsbruck, Austria, 2Agrifutur Srl, Via Campagnole 8, 25020 Afflanoello (Brescia), Italy

Traditional crops are under increasing threat from a number of subterranean pests that have proven particularly difficult to control. In central Europe these include the larvae of three Scarabs (Melolontha melolontha, Amphimallon solstitialis, Phyllopertha hortulaca), a number of different larvae of Elateridae (e.g. Agriotes obscurus, Bothynoderes punctiventris, Otiyorhynchus sulcatus), Daktulosphaira vitifoliae and the new exotic pest Diabrotica virgifera. It is not surprising that these pests are difficult to control because a major problem is their cryptic niche and the inherent difficulties of penetrating their habitat with appropriate control agents. In most of the affected agricultural systems in Europe use of chemical insecticides is undesirable or impossible. Currently the use of virulent, ecologically competent strains of insect-pathogenic fungi appears the best option. Fungal pathogens are endemic in pest populations and fulfill the key criteria of BCAs by effectiveness, autodissemmination and their excellent persistence. This paper provides examples of the successful use of the entomopathogenic Hypomyces betahyphae and Metarhizium in subterranean pest control in European agriculture, forestry and horticulture. Preventive control approaches based on the subterranean pests listed above will be discussed.

To germinate or not? Strategies of Beauveria bassiana for survival in soil
Carolyn V. Mander 1, Trevor A. Jackson 2 and Bruce Chapman 2
1Bio-Protection and Ecology Division, PO Box 84, Lincoln University, Canterbury, New Zealand, 2AgResearch, PO Box 60, Lincoln, Canterbury, New Zealand

Conidial persistence is considered important for strain selection of potential biocontrol agents but it is not clearly understood how entomopathogenic fungi maintain themselves in the soil environment. Three strains of Beauveria bassiana and one strain of B. brongniartii were compared for persistence and germination in soil. After one month, significantly higher numbers of CFUs were isolated for all strains from heat-treated soil compared with native soil. One strain (B928) exhibited greater persistence than the other strains after three months in native soil. Fluorescence microscopy was used for examination of germination and viability of conidia and showed rapid loss of viability and rapid germination for all strains in heat-treated soil. In native soil, loss of viability was reduced but still significant and germination was minimal. Interestingly, in addition to high viability, B928 showed significant germination in native soil. This study indicates that non-specific germination and saprophytic ability in soil may be linked to long-term strain survival. Differences in survival and germination between strains indicate significant genotypic variation resulting in different responses to available nutrients and/or antagonism in soil.

Variability of fitness costs associated with Cry1A resistance in Helicoverpa armigera on cotton and alternative refuge crops
Lisa Bird and Ray Akhurst
CSIRO Entomology, Canberra ACT 2611, Australia

The size and dominance of fitness costs associated with resistance have a large impact on the spread of resistance alleles. We used near-isogenic lines of Helicoverpa armigera to assess the nature of fitness costs associated with resistance to the Cry1A insecticidal proteins of Bacillus thuringiensis. Experiments were conducted with transgenic
cotton producing Cry1Ac at two stages of development in which the level of expression was significantly different and on two crops that are offered as alternatives to cotton for the mandated refuges. First instar larvae of a resistant, a susceptible and the F1, hybrid of these lines were placed individually on transgenic and conventional plants to assess their survival and development. The reproductive potential of the resulting adults was determined. These experiments showed significant variation in the nature of fitness costs associated with Cry1Ac resistance between cotton at different stages of maturity and between the conventional refuge plants. The implication of this variation for the use of refuges in resistance management will be discussed.

**STU** Contributed paper. Thursday, 8:15. **185**

*Genetic response of the spruce budworm, Choristoneura fumiferana, to sublethal Bacillus thuringiensis Cry1Ab toxin exposure*

Liliane Meunier1, Gabrielle Préfontaine1, Qili Feng2, Roland Brousseau1 and Luke Masson1

1National Research Council of Canada, Biotechnology Research Institute, Montreal, Quebec, Canada, 2Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, Ontario, Canada

Larvae of the spruce budworm (Choristoneura fumiferana) are destructive defoliators of North American forests where epidemic episodes involve major damages to spruce and balsam-fir trees. The crystal toxin Cry1Ab, which forms part of the commercial crystals produced by the entomopathogenic bacteria, Bacillus thuringiensis (Bt) strain HD-1 has been used for many years as a biological control agent against this pest. Although Cry1Ab toxins have a relatively narrow host range, toxicity is generally measured by death of the insect. Sublethal effects on non-target insects are not readily apparent but can be assessed at a molecular level. By understanding the genetic response of larvae exposed to sublethal doses of a Cry toxin, we can then proceed to assess whether genes showing altered transcriptional profiles can be used as universal Cry toxin stress markers for non-target insects. To this end, a suppression subtraction hybridization library (SSH) was created using two larval populations (control and toxin Cry1Ab treated). The transformed library was characterized by sequencing approximately 1000 clones and differential mRNA expression analysis of selected clones was assessed by quantitative-PCR. This presentation will describe the preliminary characterization of the SSH library and the identification of genes implicated in the larval stress response after low level exposure to Cry1Ab toxin.

Contributed paper. Thursday, 8:30. **186**

*Effects of entomopathogenic nematodes on the fitness cost of resistance to Bacillus thuringiensis in the pink bollworm*

Aaron J. Gassmann1, S. Patricia Stock2, Yves Carrière, and Bruce E. Tabashnik1

1Department of Entomology, 410 Forbes Building, University of Arizona, P.O. Box 210036, Tucson, AZ 85721, 2Division of Plant Pathology and Microbiology, Department of Plant Sciences, 204 Forbes Building, University of Arizona, P.O. Box 210036, Tucson, AZ 85721, USA

We evaluated effects of entomopathogenic nematodes on the fitness cost of resistance to Bt toxin Cry1Ac in the pink bollworm, Pectinophora gossypiella. P. gossypiella is a major pest of cotton in the United States. In the southwestern United States, it is currently controlled with transgenic cotton that produces Cry1Ac. Although resistance to Cry1Ac has not increased in field populations of P. gossypiella, laboratory selection has produced several resistant strains, indicating that field-evolved resistance remains a threat. In conjunction with refuges of non-Bt cotton, fitness costs can help to delay resistance. We hypothesized that the fitness cost of Bt resistance can be increased by exposure to entomopathogenic nematodes. To test this hypothesis, we compared the fitness cost of Bt resistance across several concentrations of nematodes for two hybrid populations of P. gossypiella, both of which contained Bt-resistant and Bt-susceptible individuals. We tested both Arizona-native and commercially available entomopathogenic nematodes. Our data address the extent to which incorporating entomopathogenic nematodes into an integrated pest management strategy might slow or prevent the evolution of resistance to Bt toxins.

**STU** Contributed paper. Thursday, 8:45. **187**

*Identification and characterization of Bacillus thuringiensis strains by the molecular methods*

Galina V. Kalmykova1, Ljudmila I. Burtseva1, Anatoli M. Lysenko2

1Laboratory of Insect Pathology, Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia, 2Institute of Microbiology, RAS, Moscow, Russia

The classification of Bacillus thuringiensis (Bt) strains distinguishes more than 80 subspecies. The strains of Bt can readily lose the ability to form a parasporal crystal and become similar to B. cereus. The present work deals with a large number of Bt strains of different origin. All tested strains were identified as different subspecies of Bt according to traditional taxonomy methods. DNA fingerprinting with the special DNA probe showed no polymorphism among the investigated strains of subsp. kurstaki. GC contents differed within the limits of 36-37%. Molecular DNA-DNA hybridization showed a high degree of homology equalled 95-105% among the strains of the same subspecies. The homology of total DNA among different subspecies equaled 60-80%, the most frequent being within 70%. Therefore this figure can be a real level of their DNA differences. These findings hold good for acrystalliferous mutants of the tested subspecies. It may be concluded that simultaneous molecular methods are most suited to the task of identifying strains of Bt.

**STU** Contributed paper. Thursday, 9:00. **188**

*Isolation, molecular characterization and insecticide potential of Bacillus thuringiensis strains isolated from Madurai dt. (Tamilnadu)*

A Mahalakshmi, R Shenbagarathai and K Sujatha

PG Department of Zoology & Research Centre, Madurai - 625002, Tamilnadu, India.

To isolate a naturally occurring novel Bacillus thuringiensis strain, the distribution, toxicity, morphology and gene type of B. thuringiensis from various ecological niches in Madurai, Tamilnadu was investigated. Gram-positive, endospore-forming 124 Bacillus thuringiensis like strains was isolated from 223 samples collected from agricultural and non-cultivated soils, water and dead insects. Acetate selection revealed diverse populations of B. thuringiensis. The internal transcribed spacers PCR (ITS-PCR) place the origin of the indigenous B. thuringiensis to existing B. thuringiensis (98% homology) (EMBL: AJ639659). The isolates were further grouped into seven categories based on Repetitive Palindromic PCR (REP-PCR). SDS-PAGE of the spore-crystal mixtures revealed diverse populations of B. thuringiensis which were differentiated in at least 16 distinct protein profiles. PCR Analysis using cry1, cry2, cry4 showed that the frequency of cry 4 predominated followed by cry2 and cry1. Only one isolate, named B. thuringiensis LDC-9 among seven was toxic to both Culex quinquefasciatus and Aedes aegypti (100%), was taken for further characterization and comparison with reference strains. The isolates, which belong to dipteran-active and non-toxic isolates, produced spherical crystal. These field collected isolates seem to contain new gene or genes that seem promising for biological control and resistance management. Implication of cry4 gene in toxicity is hypothesized.

Contributed paper. Thursday, 9:15. **189**

*The expansion of Bacillus thuringiensis subspp. tochunchi in environment*

Viktor P. Khodirev

Institute of Animal Systematics and Ecology, SB RAS, Novosibirsk, Russia

The search of various strain of Bacillus thuringiensis ssp. tochunchi H31 (Bt H31) was realized in different types of soil, bran and dead insect. Bt H31 was found in chernozem (1×10⁷ spore/g dried soil) and many other types of soil in Novosibirsk region. This bacterium...
Targeted delivery of genetically conjugated Cyt1Aa from *Bacillus thuringiensis* subsp. *israelensis* into myeloma model cells

Shmuel Cohen1,2, Etian Ben-Dov1, Marina Ninneitch1, Rivka Cahani2, Michael Firer2 and Arieh Zaritsky1

1Department of Life Sciences, Ben-Gurion University of the Negev, P.O.B. 653, Be'er-Sheva 84105, Israel, 2Department of Chemical Engineering and Biotechnology, College of Judea and Samaria, P.O.B. 3, Ariel 44837, Israel.

Multiple myeloma is currently an incurable cancer of plasma B cells in the bone marrow, characterized by elimination of normal blood cells, insult of the immune system and overproduction of abnormal monoclonal immunoglobulin (Ig) “M-protein”. This protein is expressed on the membrane of plasma cells and secreted into the blood. The proteolytically activated fragment of Cyt1Aa from *Bacillus thuringiensis* subsp. *israelensis* destroys cell membranes non-specifically. To treat myeloma target cells with Cyt1Aa, the lack of specificity must be overcome. This may be achieved by linking Cyt1Aa to a ligand directed to a clonotypic receptor on target cells. Various fragments of Cyt1Aa, fused genetically at the amino or carboxy terminus with a major epitope of myelin basic protein (MBP), p87-98 (VHFKNKIVTPRT), were cloned into a shuttle vector pHT315. The MBP peptide used as a ligand is recognized by the antibodies expressed on the surface of mouse tumor B cells used as a model for multiple myeloma (B-MBP). Preliminary results show that the fused protein is active against B-MBP tumor cells. This approach provides two benefits: (a) using the surface Ig as a unique receptor; (b) preventing the development of drug resistance because Cyt1Aa does not penetrate the cell but rather acts on the cell membrane.

**STU** Contributed paper. Thursday, 9:30.

**190**

**DIFFERENTIAL EXPRESSION OF CRY TOXIN IN A BACILLUS THURINGIENESIS STRAIN WITH DUAL INSECTICIDAL ACTIVITY**

Javier Torres1, Norma A. Valdez-Cruz1, Jose D. Tinoco2, Sergio Orduz2

1Unidad de Biotecnología y Control Biológico, Corporación para Investigaciones Biológicas, Carrera 72A #78B - 141, Medellín, Colombia; 2Coltabaco, Compañía Colombiana de Tabaco S. A., Autopista Sur (Carrera 50) No 5-115 Medellín, Colombia.

Transcription of cry genes is carried out during sporulation and is directed by sporulation transcription factors, and in some cases during exponential growth and regulated by vegetative specific σ factors. We studied the expression of sporulation (cry1) and vegetative (cry3) genes analysing protein expression kinetics in a wild type strain of *Bacillus thuringiensis* with natural dual toxic activity against lepidopteran and coleopteran insects. Sporulation stages were established, proteins were detected by SDS-PAGE and Western-Blot, and cry genes transcription were detected using RT-PCR. It was observed that sporulation dependent genes were transcribed during vegetative stage at a basal level followed by a cry1 mRNA increase during sporulation reaching a peak during mid sporulation phase, although the level of protein expression was low from the initial stage to sporulation onset. cry3 mRNA analysis showed that this is active during all stages studied. However, an important increased was observed during mid sporulation stage, results that corresponded to protein expression analysis. Therefore Cry3 proteins are expressed in a different pattern compared to previous reports, and Cry1 proteins also showed an atypical expression pattern. Furthermore, we observed a critical point after the Cry1 transcription (on study) during vegetative grown, because discrimination between mRNA and protein levels was found.

**STU** Contributed paper. Thursday, 9:45.

**191**

**SYMPOSIUM (Division of Bacteria). Thursday, 1:30–3:30**

**TOXIN-RECEPTOR INTERACTIONS AND MODE OF ACTION**

Vincent Vachon

Groupe d'étude des protéines membranaires, Université de Montréal, Montreal, Quebec, H3C 3J7, and Biocontrol Network, Canada

The effect of various *Bacillus thuringiensis* toxins on the permeability of the luminal membrane of *Manduca sexta* midgut columnar epithelial cells to a range of charged and uncharged solutes was monitored with an osmotic swelling assay and membrane potential measurements. Membrane permeability in the presence of a given toxin is strongly influenced by several biophysical and biochemical factors, including pH, ionic strength and divalent cations, suggesting an important role for electrostatic interactions, as well as by proteolytic enzymes. The influence of these factors can differ greatly, however, depending on the toxin being studied, even for closely related toxins such as Cry1Ac and Cry1C. Lowering temperature gradually decreased the rate of pore formation, but had little effect on the permeability of vesicles pre-incubated with toxin at room temperature. The formation of new pores, following incubation of the vesicles with toxin, could thus be almost abolished by rapidly cooling the vesicles to 2°C before the onset of the osmotic swelling experiments. Using this approach, changes in the rate of pore formation could be more easily distinguished from alterations in the properties of the pores formed, thus allowing a more detailed analysis of the kinetics and mechanism of pore formation.

**SYMPOSIUM. Thursday, 1:30.**

**192**

**INFLUENCE OF THE PHYSICO-CHEMICAL AND BIOCHEMICAL ENVIRONMENT ON THE KINETICS OF PORE FORMATION BY CRY TOXINS**

Vincent Vachon

Groupe d'étude des protéines membranaires, Université de Montréal, Montreal, Quebec, H3C 3J7, and Biocontrol Network, Canada

The effect of various *Bacillus thuringiensis* toxins on the permeability of the luminal membrane of *Manduca sexta* midgut columnar epithelial cells to a range of charged and uncharged solutes was monitored with an osmotic swelling assay and membrane potential measurements. Membrane permeability in the presence of a given toxin is strongly influenced by several biophysical and biochemical factors, including pH, ionic strength and divalent cations, suggesting an important role for electrostatic interactions, as well as by proteolytic enzymes. The influence of these factors can differ greatly, however, depending on the toxin being studied, even for closely related toxins such as Cry1Ac and Cry1C. Lowering temperature gradually decreased the rate of pore formation, but had little effect on the permeability of vesicles pre-incubated with toxin at room temperature. The formation of new pores, following incubation of the vesicles with toxin, could thus be almost abolished by rapidly cooling the vesicles to 2°C before the onset of the osmotic swelling experiments. Using this approach, changes in the rate of pore formation could be more easily distinguished from alterations in the properties of the pores formed, thus allowing a more detailed analysis of the kinetics and mechanism of pore formation.

**Symposium. Thursday, 2:00.**

**193**

**COMPARISONS OF BT TOXIN RECEPTORS AND APPLICATIONS FOR PEST INSECT CONTROL**

Michael Adang1,2, Gang Hua1, Jiang Chen1, Juan Luis Jurat-Fuentes1, and Mohd Amir Abdullah1

1Departments of Entomology and Biochemistry & Molecular Biology, University of Georgia, Athens, GA 30602, USA

Binding molecules located in the midgut epithelium are key factors in the action of *Bacillus thuringiensis* (Bt) toxins. Cadherins are primary receptors for Cry1A toxins in Lepidoptera because of their high affinity for Cry toxins, ability to catalyze toxin-induced cell death and “knockout” mutations result in resistance. Although there is significant homology between cadherins from different lepidopterans, each cadherin has different affinity for specific Cry toxins. Our data indicate that toxin-binding cadherins are localized to the tips and “hammock” regions of microvilli in brush border epithelium. Aminopeptidases and membrane-bound alkaline phosphatases that bind toxin, are anchored by glycosylphosphatidylinositol which is presumably related to their localization in lipid rafts, and are primarily distributed along microvilli in the posterior region of *Manduca sexta* larvae. Glycolipids are receptors for Bt toxins in nematodes and insects. Loss of glycolipid carbohydrates causes toxin resistance in *Caenorhabditis elegans*. In both nematodes and insects glycolipids specifically bind Bt toxins. The role of binding proteins in toxin action has evolved from arguments about whether aminopeptidases or cadherins are functional receptors to a model that integrates multiple binding proteins and even glycolipids into toxin action. The implications of multiple receptors and their toxin specificities for pest insect control will be discussed.
and conservation biology for all organisms on earth, including

Estimation of species geographic distribution is critical to biodiversity
diversity. For example, recent developments in geographic

Insecticidal Cry proteins undergo conformational changes from a
monomeric structure to a prepore-oligomeric form that is membrane
insertion competent. We have characterized the structural and
functional changes of Cry toxins upon oligomerization and membrane
insertion. We studied the stability of these three structures after urea
and thermal denaturation by monitoring intrinsic tryptophan
fluorescence of the protein and 1-anilino naphthalene-8-sulfonic acid
(ANS) binding to unfolded proteins. Our studies suggest that a more
flexible conformation could be necessary for membrane insertion and
this flexible structure is obtained by toxin oligomerization (Biochemistry 2004, JBC 2004). Finally we will present data
regarding the interaction of the pre-pore with the second receptor
(APN) by using antibodies that specifically recognize the Cry1Ab
oligomeric structure and affects this interaction. Our data suggests that at least two receptor molecules, cadherin and APN, are
sequentially involved in the interaction of Cry 1 toxins with its target
membrane.

Symposium. Thursday, 3:00. 195
Toxin binding site of the Heliothis virescens cadherin
Ruiyu Xie 1, Meibao Zhang 2, Linda S. Ross 1, Isabel Gomez 1, Karlygash Aimanova 1, Daniela I. Oltean 1, Alejandra Bravo 2, Mario Soborón 1 and Sarjeet S. Gill 2
1Department of Cell Biology and Neuroscience, University of California, Riverside, CA92521, USA
2Instituto de Biotecnología. Universidad Nacional Autónoma de México, Cuernavaca 62250 Morelos, México

Bacillus thuringiensis Cry1A proteins exerts their toxic effect through a
receptor-mediated mechanism, involving both cadherin and
aminopeptidases. Both of these proteins have been identified in a
number of insects including Heliothis virescens and Manduca sexta. Disruption of both cadherin and aminopeptidase affects Cry1A
Toxicity. In a recently reported study we show that the Cry1A toxin-
binding region in H. virescens cadherin-like protein was mapped to a
40-amino acid fragment, from aa 1422 to 1461. Mutations in this
region, to which the Cry1A binds through its loop 3, resulted in the
loss of toxin binding. Further, feeding of the anti-H. virescens
cadherin antisum or the partial cadherin-like proteins, which contain the
Toxin binding region, in combination with Cry1A alleviated insect mortality, showing this region is involved in insect
toxicity. Immunohistochemistry showed the cadherin-like proteins are
present in the insect mid gut apical membrane, which is the target site
of Cry toxins. This subcellular localization is distinct from that of
classical cadherins, which are usually present in cell-cell junctions.

Symposium. Thursday, 1:30. 196
Biogeographic distribution and diversity of entomopathogenic
nematodes: Natural patterns or human- biased trends?
S. Patricia Stock
Department of Plant Sciences-Department of Entomology, University of Arizona. Tucson, AZ 85721, USA

Estimation of species geographic distribution is critical to biodiversity
and conservation biology for all organisms on earth, including
nematodes. Many geographic applications have been developed in
recent years that offer new possibilities for understanding biological
diversity. For example, recent developments in geographic
information (GIS) and global positioning (GPS) systems, remote
sensing and ecological modeling have opened doors to exciting new
synthetic analyses in conservation biology. Moreover, these tools
have been applied in nematology to assess distributional patterns and
levels of damage of several plant-parasitic nematodes to help make
predictions for future control management strategies. However,
exploration of these possibilities for predicting species distributions
of entomopathogenic nematodes (EPN) (Steinernematidae and
Heterorhabditidae) is yet not possible as most biodiversity data of
EPN is fragmented. In most instances, sampling has rarely been
planned in a systematic manner, and has mostly been limited to
countries or geographic regions where experts in the field are settled.
In this presentation I will review the current state of EPN diversity
and geographic distributional patterns. Potential solutions and/or
strategies to expand our current knowledge on this subject will be
discussed.

Symposium. Thursday, 1:45. 197
Relating entomopathogenic nematode presence and abundance to
habitat variation in an agroecosystem
Casey Hoy, Parwinder Grewal, Ganpati Jagdale, Nuris Acosta and
Janet Lawrence
Department of Entomology, The Ohio State University, Ohio
Agricultural Research and Development Center, Wooster, OH 44691, USA

If knowledge of environmental conditions associated with naturally
occurring entomopathogenic nematode populations is sufficient, then
crop management practices might be designed to encourage presence
and persistence of these biological control agents and long-term pest
suppression. We have focused our research on naturally occurring
entomopathogenic nematode populations in a vegetable production
area in Ohio with a diverse mixture of crops, and insect pests, and
high organic matter soils. A systematic survey of 600 sampling sites
representing 6 different habitat classes in and around the production
area identified 41 sites that had endemic steinernematid or
heterorhabditid populations. The habitat class of the positive sites
varied, 22 were in grassy field borders within the vegetable
production area, and the remainder were outside of the production
area: 10 in forest, 4 in residential lawns, 3 in field crops (corn and
soybeans), 2 in successional shrub land, and none from within
the vegetable fields. Results of a multivariate statistical analysis on soil
food web structure, based on free-living nematodes in soil samples,
and soil physical and chemical properties at each of the sites will be
discussed with respect to their relative importance in explaining
presence and abundance of these endemic entomopathogenic
nematode populations.

Symposium. Thursday, 2:00. 198
Host finding and infection decisions in the soil
1Department of Nematology, University of California, Davis, Davis,
CA 95616, 2Monsanto Company, 700 Chesterfield Pkwy W,
Chesterfield, MO 63017, 3USDA-ARS, SAA, SE Fruit and Tree Nut
Research Unit, 21 Dunbar Road, Byron, GA 31008, ‘USDA ARS
GMRC, 1515 College Ave, Manhattan, KS 66502, USA

Entomopathogenic nematodes (EPNs) search through the soil for hosts.
They respond to cues produced by hosts and make infection
decisions based upon these cues. Cues vary based upon host species
and infection status. EPNs respond most strongly to host species that
support high levels of reproduction. More recent work suggests that
EPN infective juveniles (IJJs) respond to infected hosts differently
than non-infected hosts. We now ask how infection decisions are
linked with fitness when an IJ is presented with the opportunity to
join an ongoing infection. We have measured the temporal course of
infection decisions of three EPN species in two host species. The rate
of infection varies both with EPN species and with the relative
permissiveness of the host. EPN IJs continue to join conspecific
infections almost until IJs begin to emerge from the host, albeit at
rates that gradually decline as the infection progresses. However,
when presented with the chance to join heterospecific infections, *S. glaseri* will infect whereas *S. carpocapsae* will not. When hosts were held until IJ emergence, *S. glaseri* was a superior competitor inside the host, reinforcing their decisions to enter heterospecific infections. Thus, the link between fitness and infection decisions is again supported.

**Symposium. Thursday, 2:15. 199**

Formulations and methods for enhancing post-application survival

David L. Shapiro-Ilan

USDA-ARS, Southeast Fruit and Tree Nut Research Lab, Byron, GA 31008, USA

Post-application survival of entomopathogenic nematodes is a key factor affecting field efficacy. Formulation and application technology can play a major role in determining survivability. Various formulations have been developed for soil application; a number of these are currently in use. The utility of entomopathogenic nematodes may be expanded through some recently developed formulations that offer greater potential for survival in above-ground applications. Formulation or application of nematodes in their infected hosts may also offer opportunities for enhanced survival. Entomopathogenic nematode species or strain selection can have considerable impact on post-application survival. Indeed in recent laboratory experiments conducted in soil, we observed substantial variation in survivability of more than 20 entomopathogenic nematode strains and species.

**Symposium. Thursday, 2:30. 200**

Abiotic factors affecting success of entomopathogenic nematodes in the field

Lawrence A. Lacey

USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA

A wide variety of abiotic factors can affect the ability of infective juveniles (IJ)s of entomopathogenic nematodes to find and infect insect hosts and survive in agroecosystems. Among environmental factors, temperature and moisture (relative humidity and free water) are the best studied and are the most influential factors that limit or enhance activity of IJ. Their effects are most pronounced on foliage and other exposed habitats. The efficacy of one of the most widely utilized EPN species, *Steinernema carpocapsae*, begins to decline at 15°C and comes to a complete standstill at 10°C. Species such as *S. feltiae*, *S. kraussei*, and some of the heterorhabditid species are active at lower temperatures (5-10°C), but may be inhibited at higher temperatures. The opposite is the case for *S. riobrave*. Adequate moisture is critical for the movement and survival nematodes. Water applied after application of IJs is also needed to help penetrate barriers (foliage, thatch, etc.). Exposure to ultraviolet light and extremes of pH can also affect IJ survival. Abiotic factors in the application of IJs (friction and pressure) can have detrimental effects on the viability and vigor of IJs. Small nematodes, such as *S. carpocapsae* and *Heterorhabditis bacteriophora* can withstand spray system pressures up to 2000 kPa whereas larger species, such as *H. megidis*, the pressures within the spray system should not exceed 1380 kPa. The friction produced within the spray system, particularly the nozzles, swirl plates and filters could also have detrimental effects on IJs. Physical barriers and agrochemicals and other agricultural practices can also influence IJ infectivity and survival.

**Symposium. Thursday, 2:45. 201**

Biotic factors and farming systems affect persistence and recycling of EPN

Mary E. Barbercheck and Randa Jabbour

Department of Entomology, The Pennsylvania State University, University Park, PA 16802, USA

Soil is home to a complex assemblage of micro- and macroorganisms that interact with endemic and applied entomopathogenic nematodes (EPN). Agricultural practices (e.g., frequency and intensity of soil disturbance, amount of plant residue left on the soil surface, agrochemical use, crop species and variety) can profoundly affect the biotic and abiotic soil environment (e.g., temperature, moisture, abundance and diversity of soil organisms, plant diversity, host availability). We will review research results on the effects of environmental conditions created by production practices in various agricultural systems on the occurrence of natural populations and persistence of applied populations of EPN. We will also report recent findings on the effects of agricultural systems on the occurrence of endemic EPN.

**Symposium. Thursday, 3:00. 202**

Recycling and long-term persistence of entomopathogenic nematodes

Albrecht M. Koppenhöfer

Dept. Entomology, Rutgers University, Blake Hall, 93 Lipman Dr., New Brunswick, NJ 08901, USA

Entomopathogenic nematodes can recycle in infected hosts and the emerging nematode progeny may provide additional control of a present insect problem but will also prolong the persistence of the nematode population, ideally to have an impact on following pest generations. I will discuss observations made on long-term effects of entomopathogenic nematodes for various insect and nematode combinations, particularly using the example of *Steinernema scapterisci* and *S. scarabaei*. *S. scapterisci* has been studied extensively as a control agent for introduced mole crickets, *Scapteriscus spp.*, in the southeastern USA. It frequently becomes established after applications in turfgrass and particularly pasture situations. *S. scapterisci* can be spread over longer distances by infected adult mole crickets. *S. scarabaei* has shown exceptional potential as a curative control agent and as a long term suppressant of white grubs. Applied for the management of larval oriental beetle, *Anomala orientalis*, populations in turfgrass, *S. scarabaei* (0.1 - 2.5 × 109 *S. scarabaei* ha) has already persisted for 2.5 years after application, providing 50 - 100% *A. orientalis* control at 1 month after application (MAT), 96 -100% in the following spring (8 MAT), 62 -91% at 13 MAT (following grub generation), and 31 -94% at 25 MAT.

**Symposium. Thursday, 3:15. 203**

Ecology of entomopathogenic nematodes: Past, present, and future

Harry K. Kaya

Department of Nematology, University of California, Davis, CA 95616, USA

Ecological studies with entomopathogenic nematodes have been ongoing since their initial discovery in the late 1920s. Most early ecological studies had an applied aspect with emphasis on nematode (i.e., *Steinernema glaseri*) persistence after field application for control of Japanese beetle larvae. In the 1980s, persistence studies continued with the commercial development of several nematode species, but it became increasing important to study the behavior of these nematodes and their interactions with other organisms in the environment. Accordingly, laboratory studies on nematodes in response to abiotic (moisture, temperature, soil type) and biotic (antagonists, host cues, other entomopathogens) factors were conducted. Dispersal studies showed that nematode species had different behaviors which led to the findings that some nematode species had a sit-and-wait, an intermediate, or a widely foraging strategy. These results demonstrated that the right nematode species had to be applied against a particular pest species. However, much of the research has been focused on only a handful of nematode species and the ecology and behavior of other species need to be elucidated. In addition, although some basic field studies with these nematodes have been conducted, a greater emphasis on population dynamics and factors that initiate nematode epizootics in insect populations is needed.
VIRUSES 5

Contributed paper. Thursday, 1:30. 204

On the analogy of the baculovirus and whispovirus DNA binding proteins
Marcel Westenberg, Jeroen Witteveldt, Era Tuladhar,
Mozes F. Boyong, Just M. Vlak

Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

The baculovirus DNA-binding proteins P6.9 are involved in the condensation and packing of the dsDNA genome. These proteins are rich in arginines and serines, but differ in length among virus species and their phosphorylation status is important for DNA (un)coating. The binding of P6.9 to DNA is sequence specific, but specificity may be brought in through the interaction between P6.9 and other viral proteins in the virus assembly. To investigate this aspect a p6.9null Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) bacmid was generated, which upon transfection into Sf21 cells did not lead to production of infectious virus particles. This defect was rescued by the insertion of the Spodoptera exigua (SeMNPV) p6.9 gene into the bacmid. However when the DNA binding protein gene vp15 of the unrelated White Spot Syndrome Virus (WSSV) was inserted, no infectious virus particles could be produced. Despite the presence of predicted phosphorylation sites, phosphorylated VP15 could be detected neither in WSSV-infected shrimp tissue nor in baculovirus-infected insect cells. Thus, either DNA condensation and packing relies on different mechanisms in the different virus families or, in contrast to SeMNPV P6.9, WSSV VP15 is unable to have a specific interaction with some AcMNPV proteins involved in viral assembly.

Contributed paper. Thursday, 1:45. 205

Replication in Trichoplusia ni larvae of AcMNPV mutants that express only IE0 or IE1
Martin A. Erlandson1, Taryn M. Stewart2, Leslie G. Willis3, and David A. Theilmann4

1Saskatoon Research Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon S7N 0X2, Canada, 2Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C. V0H 1Z0, Canada, 3Faculty of Agricultural Sciences, University of British Columbia, Vancouver, B.C. V6T 1Z4, Canada

The ie0-ie1 spliced gene complex of baculoviruses is essential for replication of the archetype baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV). Our previous studies showed that IE0 may be more important for early infection events than IE1. In Spodoptera frugiperda (Sf9) tissue culture cells viruses lacking both ie0 and ie1 are unable to replicate showing that the gene complex is essential. The objective of this study was to determine role of ie0 and ie1 on the infection process in live insects using the caterpillar Trichoplusia ni. AcMNPV bacmids repair viruses were constructed that expressed only IE0 or IE1, or both proteins. Polyhedra produced in vitro and in vivo (T. ni larvae) were used in single dose time-to-mortality bioassays. Results show that viruses expressing only IE0 were significantly delayed for the onset of insect death but cumulative mortality levels eventually approach WT levels. Viruses expressing only or predominately IE0 were significantly impaired for time to death. Viruses expressing both IE0 and IE1 but at expression level ratios different from WT also were significantly impaired. These results support the conclusion that the expression of IE0 and IE1 at the correct time and in the right levels is essential for a successful infection.

Contributed paper. Thursday, 2:00. 206

A common network for the activation of early promoters through baculovirus polyhedrin upstream sequence
Carol P.-Y. Wu and Yu-Chan Chao

Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 115, Taiwan, ROC

Baculovirus is a widely used tool for recombinant protein production. Previously we identified a novel polyhedrin upstream (pu) sequence, which can activate many early promoters for high-level foreign protein production with the co-infection of baculovirus, AcMNPV, 2002. Novel baculovirus DNA elements strongly stimulate activities of exogenous and endogenous promoters. J. Biol. Chem. 277:5256-5264). In order to identify viral genes for pu activation, all genes in the entire 130kb baculovirus genome were screened. Our results showed that a set of genes, including ORF147, ORF151, and ORF153, were required for the strong stimulation of pu sequence, with or without the strong enhancer, the homologous region (hr) sequence of baculovirus. Interestingly, we later found that these three ORFs are the same as those needed to activate p143 promoter previously (Lu and Carstens. 1993). Immediate-early baculovirus genes transactivate the p143 gene promoter of Autographa californica nuclear polyhedrosis virus. Virology 195: 710-718). Further experiments were thus carried on, and found that although these three ORFs can activate the promoter of p143, further addition of pu sequence can significantly enhance the expression of this promoter. Thus, a shared network in the genome of the baculovirus for the strong activation of early promoters through pu and ORFs 147, 151, and 153 is found.

Contributed paper. Thursday, 2:15. 207

Functional characterization of BmNPV and SeMNPV late gene transcription and genome replication factors in the insect cell line SF-21
Marcelo F. Berretta, Mandar Deshpande, and A. Lorena Passarelli

Division of Biology, Kansas State University, Manhattan, KS 66506-4901, USA

We are interested in assessing the functionality of late gene transcription and DNA replication machineries of closely related and more divergent baculoviruses in different cell lines. It has been well established that 19 AcMNPV late expression factors (lef(s)) stimulate substantial levels of late gene promoter activity in SF-21 cells. Thus, we constructed two sets of clones containing either the Bombyx mori nucleopolyhedrovirus (BmNPV) or the Spodoptera exigua NPV (SeMNPV) epitope-tagged homologs of the AcMNPV lef(s) under control of the constitutive Droso phila heat shock 70 protein promoter and tested their ability to activate an Autographa californica NPV (AcMNPV) late promoter-reporter gene cassette in SF-21 cells. We tested the potential of individual or predicted functional groups of BmNPV or SeMNPV lef(s) to successfully replace the corresponding AcMNPV gene(s) in transient late gene expression assays. We found that most, but not all, BmNPV lef(s) were able to fully or partially substitute for the corresponding AcMNPV homolog in the context of the remaining AcMNPV lef(s). However, only the SeMNPV lef-5 was able to substitute for the AcMNPV lef-5 in this functional assay. Finally, we also tested the ability of BmNPV, SeMNPV, and AcMNPV origins of DNA replication to support late gene transcription.

Contributed paper. Thursday, 2:30. 208

Oral infection of Spodoptera exigua larvae with an AcMNPV mutant lacking the apoptosis suppressor p35
Rollie J. Clem1, Louis Heaton1, and Miriam Burton2

1Division of Biology and 2Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, USA

We have previously found that a mutant of AcMNPV lacking the p35 gene is less infectious in late instar S. exigua larvae than a control revertant virus when the virus is administered by intrahemocoelic injection, and apoptosis is prevalent in the infected tissues of p35 mutant-infected larvae. In this study we infected late instar S. exigua larvae by the oral route with p35 mutant and control revertant viruses expressing GFP, and we monitored the infection using scanning confocal microscopy to visualize infected cells and TUNEL staining to visualize apoptotic cells. S. exigua larvae were found to be at least 100-fold more resistant to oral infection with p35 mutant AcMNPV than to infection with revertant virus. In S. exigua larvae infected with the p35 mutant, apoptosis was clearly observed during secondary
infection in tissues such as tracheal epithelium and fat body. This secondary infection phenotype was very similar to what was observed previously following infection by intrahemocoelic injection. However, apoptosis was not observed in primary infections of the midgut epithelium. These results suggest that the cellular response of midgut epithelium to p35 mutant infection is different from that of other tissues in the insect.

Contributed paper. Thursday, 2:45.

Identification and functional analysis of Leucania separata multiple nuclear polyhedrosis virus iap3 and p49 genes in s9 cells
Enqi du, Weixin Jin, Wenke Zhou, Feng Yan, Yipeng Qi
Institute of Virology, Wuhan University, Wuhan 430072, People's Republic of China

Leucania separata multiple nuclear polyhedrosis virus (LsMNPV) genome was firstly sequenced by our laboratory in 2002. LsMNPV possessed two types of antiapoptotic genes, iap and p49. The iap gene also includes three members that were designated as ls-iap1, ls-iap2, and ls-iap3. The structure and amino acid sequence homology with other baculoviruses related genes prove that ls-iap3 was most related to functional opia3 (44%), while ls-p49 was only 28% homology with reported sl-p49. Ls-p49 also contained a reactive-site loop (RLS), while had different predicted cleavage site (KKLDDG or SATD87 E ) from sl-p49 (TVTD94 G). Functional analysis of ls-iap3 and ls-p49 by transient assay in S9 cells revealed that both ls-iap3 and ls-p49 block apoptosis induced by actinomycin D and rescued replication of p35 deficient-mutant AcMNPV. The results showed Lsiap3 and Lsp49 were both functional apoptotic suppressor in s9 cells. However, whether ls-iap3 or ls-p49 or both with anti-apoptotic functions in S1 cell is under-studying.

Contributed paper. Thursday, 3:00.

Characterization of a novel entomopoxvirus homolog of baculovirus P35
John C. Means and Rolielle J. Clem
Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66506, USA

We have identified a gene from Ansaecta moorei entomopoxvirus (AmEPV) with low but significant homology to baculovirus p35 genes. This gene, previously designated ORF AMV010, is predicted to encode a protein of 32.7 kD of predicted molecular weight. In keeping with previous nomenclature we have re-named the gene AMVP33. When ectopically expressed, AMVP33 blocked apoptosis in insect and human cell lines. Recombinant P33 was purified and tested for its ability to directly inhibit Drosophila caspases. P33 was found to be a stoichiometric inhibitor of the effector caspases DrICE and DCP-1 but was only able to inhibit the apical caspase Dronc when infected with AcMNPVAp35k/pol+. And the transient ORF390 expression allowed AcMNPVAp35k/pol+ replication in S9 cells and polyhedra formation. Consistently, SF9 cells stably expressing ORF390 protected the cells from cell death induced by actinomycin D. Furthermore, we demonstrated that ORF390 is a caspase substrate inhibitor with a P35-like mechanism and also has the capacity to block apoptosis in Hela cells. These data suggested that ORF390 of WSSV represents a novel antiapoptotic gene involved in apoptosis regulation.

Insect expression systems, gene therapy and vaccine development

Symposium. Thursday, 4:00.

Protein N-glycosylation in the baculovirus-insect cell system.
Donald L. Jarvis, Jared J. Aumiller, and Jason R. Hollister
Department of Molecular Biology, University of Wyoming, Laramie, WY, USA

Insect protein glycosylation pathways appear to be intermediate in complexity relative to lower eucaryotes, such as yeasts, and higher eucaryotes, such as mammals. Insects generally appear to perform the same early steps in protein N-glycosylation, including N-glycan assembly, transfer, and trimming, as lower and higher eucaryotes. However, they generally appear to lack some of the functions required for N-glycan elongation. As a result of this fundamental difference in insect and mammalian cells, recombinant N-glycoproteins produced using the baculovirus-insect cell system typically lack the complex, terminally sialylated glycans of many native mammalian glycoproteins. Instead, insect-derived products typically have praeimannose N-glycans at the sites occupied by complex, terminally sialylated N-glycans in native mammalian N-glycoproteins. We have been addressing this problem by using metabolic engineering methods to extend the protein N-glycosylation pathway of lepidopteran insect cell lines. These efforts have yielded transgenic insect cell lines that encode and express mammalian glycosyltransferases and enzymes involved in CMP-sialic acid biosynthesis. Relative to the parental lines, these new cell lines can still serve as hosts for baculovirus expression vectors and can produce similar levels of recombinant glycoproteins. Unlike the parental insect cell lines, however, the transgenic lines can produce recombinant glycoproteins with complex, terminally sialylated N-glycans. This talk will focus on the creation and characterization of these transgenic insect cell lines.

Symposium. Thursday, 4:30.

Densovirus-derived vectors for stable expression of foreign proteins in insect cells and somatic transformation of insects
Max Beroin
Laboratoire de Pathologie Comparée des Invertébrés EPHE, UMR 1231 Biologie Intégrative et Virologie des Insectes, Université Montpellier II, 34095 Montpellier, France

The availability of plasmids containing infectious densovirus (DNV) genomes and of DNV transcription maps has prompted research into their potential as expression vectors. A series of non-infectious vectors derived from the Junonia coenia densovirus (JcDNV) have been constructed expressing non selectable (lacZ) or selectable markers (neo, gfp) inserted in frame into the VP gene under control of the P9 promoter. By transfecting these constructs to lepidopteran cell lines, cell clones stably expressing the transgene could be produced. Analysis of transformed cells revealed that the JcDNV sequence was integrated into the host cell DNA and that the 5’ inverted terminal
repeat region was the primary site of recombination. Other JcDNV-derived constructs were made expressing genes of interest such as human erythropoietin and human γ interferon. Sf9 cell clones constitutively expressing these genes were obtained and the biological activity of recombinant proteins was demonstrated. By micrococcal neejecting Drosophila melanogaster preblastoderm eggs with a JcDNV-derived plasmid expressing the lacZ gene, high β-galactosidase expression was observed in somatic tissues throughout ontogenesis, from larvae to adult flies. JcDNV-derived vectors thus appear as interesting tools for somatic transgenesis. Taken together these results demonstrate the flexibility and reliability of using denvirus-derived vectors for multiple biological applications.

Symposium. Thursday, 5:00. 214

BacMam viruses: Versatile tools for mammalian cell-based assay development
J. Patrick Condreay
Department of Gene Expression and Protein Biochemistry, GlaxoSmithKline Discovery Research, Research Triangle Park, NC 27709, USA

Recombinant baculoviruses modified to contain mammalian cell-active expression cassettes have been shown to deliver their DNA and mediate gene expression in mammalian cells. We have demonstrated that these BacMam viruses will transduce a variety of established and transformed cell lines and primary cells without overt deleterious effects. Gene expression in transfected populations is modulated by variation of viral multiplicity, or use of inhibitors of histone deacetylase. The virus does not replicate in mammalian cells and is rapidly inactivated by serum complement giving it a favorable biosafety profile compared to other recombinant viral vectors. Multiple viruses can be used to deliver different subunits of multi-component assays. The advantages and flexibility of this system make it an excellent enabling tool for mammalian cell-based assay development. Gene delivery is accomplished with simple liquid addition steps making the BacMam system compatible with automated high throughput screening platforms. We have used these viruses to configure robust, reproducible multi-well assays for G protein-coupled receptors, ion channels, nuclear receptors, and membrane transporters. Libraries of viruses can be easily generated and stored for delivery to appropriate cells in a variety of formats, providing a versatility in assay configuration not matched by usual paradigms such as stable cell lines.

Symposium. Thursday, 5:30. 215

Tailoring the baculovirus insect cell expression system for the production of subunit vaccines
Monique M. van Oers, Stephen A. Kaba and Just M. Vlak
Laboratory of Virology, Wageningen University, Wageningen, The Netherlands

The baculovirus-insect cell expression system is widely used for the production of recombinant proteins. Membrane proteins, likely candidates for subunit vaccines against enveloped viruses and protozoan parasites, are often more troublesome to produce than cytoplasmic proteins. A protein expressed at low levels and in non-native forms is the Thelidia parva sporozoite surface protein p67. T. parva is a protozoan parasite which causes the fatal cattle disease East Coast fever. Different parts of p67 were produced as fusions to the p67 signal peptide with the honeybee melittin signal surface and recombinant p67 and with a single boost immunization. By replacing the p67 signal peptide with the honeybee melittin signal surface and by removing its transmembrane domain, a secreted form was obtained. The integrity of this secreted protein was further confirmed by deleting the chitinase and v-cathepsin genes from the baculovirus vector. These deletions were performed in a bacmid setup, allowing broad scale application of this novel vector.

Contributed paper. Thursday, 4:00. 216

New Bacillus sphaericus toxin genes in strains able to overcome binary toxin resistance in Culex larvae
Colin Berry and Gareth W. Jones
Cardiff School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 1US, UK

Bacillus sphaericus strains may produce the highly potent mosquitoicidal binary toxin (Bin) on sporulation and such strains are of great value in mosquito control programmes worldwide. However, instances of Bin resistance have been reported in Culex larvae that may reduce the effectiveness of control. More recently (1,2) several strains have been reported that are able to overcome Bin resistance and a spore associated protein of 49kDa (P49) has been identified as a candidate toxin. We have cloned the gene encoding P49 and a further putative toxin from B. sphaericus strain 1A859 and will report their characterisation and possible role in mosquitoicidal activity.


Contributed paper. Thursday, 4:15. 217

Toxicity and synergy of Mtx-1 and Mtx-2 toxins from Bacillus sphaericus against susceptible and resistant lines of Culex quinquefasciatus
Margaret C. Wirth1, Colin Berry2, Yangkun Yang2, William E. Walton1, and Brian A. Federici1,3

1Department of Entomology, University of California, Riverside, CA 92521, USA, 2Cardiff School of Biosciences, Cardiff University, Cardiff, Wales, UK, 3Interdepartmental Graduate Programs in Microbiology and Genetics, Genomics, and Bioinformatics, University of California, Riverside, CA 92521, USA

In addition to the mosquitoicidal binary toxins (Bin A, Bin B) produced at sporulation, certain highly active Bacillus sphaericus strains produce lethal proteins such as Mtx toxins during vegetative growth. Mtx-1 is a 100-kDa protein with regional homology to ADP-ribosyl transferase bacterial toxins, whereas Mtx-2 shows homology to pore-forming toxins of Clostridium perfringens and Pseudomonas aeruginosa. Because the mode of action of Mtx toxins differs from that of Bin toxins, their interaction with Bin and other mosquitoicidal bacterial toxins was tested. Lyophilized powders of recombinant E. coli expressing Mtx-1 or Mtx-2 proteins were bioassayed, alone or in combination with unrelated insecticidal toxins, for activity against Culex quinquefasciatus that were either susceptible to B. sphaericus and B. thuringiensis subsp. israelensis toxins, or resistant to one or more of these toxins. Both Mtx powders showed moderate activity (LC50, 3 µg/ml) against susceptible and resistant mosquitoes. When Mtx-1 or Mtx-2 and B. sphaericus were combined in a 1:3 ratio (wt/wt), the toxicity of the mixture was enhanced against susceptible and B. sphaericus-resistant mosquitoes and resistance was suppressed from >10,000-fold to 25 - 75-fold through synergy. Toxin synergy is a widespread phenomenon in highly active mosquitoicidal bacteria, and therefore the mechanism(s) of synergy are key to understanding their activity.

Contributed paper. Thursday, 4:30. 218

Pore-forming determinants of Bacillus thuringiensis Cry4 mosquito-larvicidal proteins
Chanun Anguswthanaomhajh
Laboratory of Molecular Biophysics and Structural Biochemistry, Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakornpathom 73170, Thailand

It was initially demonstrated that helices 4 and 5 of the Cry4Aa mosquito-larvicidal protein from Bacillus thuringiensis subsp. israelensis (Bi) this toxic determinant against Aedes aegypti larvae, likely being involved in membrane-pore formation. Further analysis revealed a crucial role in toxicity for the positively charged side-chain of Arg-158 in helix 4, conceivably involved in the passage of ions.
through the pore. The highly conserved Asn-183 in helix 5 was also found to be important for Cry4Ba toxin function. Directed mutations within the alpha4-alpha5 loop of Cry4Ba revealed that polarity of Asn-166 and highly conserved aromaticity of Tyr-170 are critically involved in larvicidal activity. A crucial role in toxicity was also revealed for the conserved aromatic residue at position 202 within the alpha4-alpha5 loop of the closely related Bti mosquito-larvicidal protein, Cry4AA. Interestingly, both the proline-rich motif (P193PNP196) and the unique disulphide bond (C192-C199) in the alpha4-alpha5 loop were found to be structurally essential for Cry4AA toxicity, possibly required for efficient penetration of the alpha4-alpha5 transmembrane hairpin into the lipid membrane. HPLC purified Cry4Ba alpha4-alpha5 hairpins were shown to be highly capable of inducing liposome permeability, constituting the region responsible for pore formation within the toxin molecule. Unrestrained molecular dynamics (MD) simulations performed with a modelled pore comprising six copies of the Cry4Ba alpha4-alpha5 hairpin or its derivatives placed in solvated lipid membrane bilayers (POP/C/water) suggested that mutations at the critical Arg-158 residue affect structural integrity of the toxin-induced pore. Consistent with the reduced conductances observed for single channels formed by biological inactive N166 mutant toxins, MD simulations of the Cry4Ba-pore model also revealed a significant decrease in the extent of hydrogen bonding at position-166 with water molecules when Asn-166 was substituted with Ile, suggesting that Asn-166 is conceivably involved in ion conduction of the Cry4Ba toxin.

Contributed paper. Thursday, 4:00. 219
Host plant determines efficacy of Beauveria bassiana against western flower thrips
Todd A. Ugine1, Stephen P. Wright2, John P. Sanderson1
1Department of Entomology, Cornell University, USA, 2USDA-ARS, PPRU, US Plant Soil and Nutrition Laboratory, USA
Multi-trophic interactions among the host plant, insect pest and insect pathogenic fungi are among the most important yet poorly studied biotic determinants of efficacy. Dosage-response assays evaluated the effects of rearing host plant and assay host plant, kidney bean versus garden impatiens in each instance, on efficacy of Beauveria bassiana (strain GHA) against the western flower thrips. Insects reared on the two host plants were exposed continuously to fungus-inoculated bean or impatiens leaf disks and percent mortality assessed after five days. Thrips maintained on bean foliage were 7-40 times more susceptible to B. bassiana infection than thrips maintained on impatiens foliage. The slopes of the probit regression lines were significantly higher for thrips maintained on beans compared to impatiens, irrespective of the rearing host plant (slopes of 1.6 and 1.4 on beans versus 0.8 and 0.6 on impatiens). A second assay was conducted to determine what effects exposure host plant and length of exposure to bean and impatiens foliage had on fungal efficacy. Thrips were exposed to B. bassiana-inoculated bean or impatiens foliage and reciprocal transfers to the alternate host were made at three time intervals, 12h 24h and 48h. Percent mortality was assessed after five days. There was a significant 15% reduction in percent mortality of thrips exposed to fungus on impatiens foliage, but no effect of exposure time.

Contributed paper. Thursday, 4:15. 220
Beauveria bassiana and Fusarium oxysporum as endophytes in banana tissue culture plants
Thomas Dubois1, Clifford S. Gold1, Pamela Paparu1, Juliet Akello1, Ekwamu Adipala2, and Daniel Coyne1
1International Institute of Tropical Agriculture, Southern and Eastern Africa Regional Centre, Namulonge, P.O. Box 7878, Kampala, Uganda, 2Department of Crop Science, Makerere University, P.O. Box 7062, Kampala, Uganda
Among the major constraints to highland cooking banana in Uganda is the high level of nematodes and banana weevils. Naturally-occurring endophytes such as Fusarium oxysporum are antagonistic to these pests. Control by endophytic F. oxysporum can be greatly enhanced when artificially inoculated. We contrasted inoculation through root and corm dipping using a spore suspension with inoculation using a solid substrate. The use of a solid substrate inoculation method resulted in the highest root colonization. No differences among inoculation methods were observed with respect to corm colonization. Corms were colonized to a higher extent than roots but hyphal density in the roots was higher than in the corms. Root persistence of endophytic F. oxysporum was sustained for up to 25 weeks. In contrast, corm tissue colonization decreased rapidly. Although corms are initially colonized to a higher extent than roots following inoculation, hyphal density is much lower, presumably allowing other microbes to occupy available niches and explaining the difference in persistence between roots and corms. When tissue culture plants are inoculated with Beauveria bassiana using a root and corm dip method, percentage colonization was 47.3% after four weeks, demonstrating that B. bassiana can be used as an artificial endophyte in banana.

Contributed paper. Thursday, 4:30. 221
Microbial control of the banana weevil, Cosmopolites sordidus, with Beauveria bassiana
Clifford S. Gold1, Caroline Nankinga1,2, William Tinzara1, Thomas Dubois1, Juliet Akello1, and Willerforce Tushemereirwe2
1International Institute of Tropical Agriculture, Southern and Eastern Africa Regional Centre, Namulonge, P.O. Box 7878, Kampala, Uganda, 2Uganda National Banana research Programme, NARO, P.O. Box 7065, Kampala, Uganda
The East African highland banana is the principal staple crop of the Great Lakes region of eastern Africa. The banana weevil, Cosmopolites sordidus, is the most important insect pest of highland banana. The larvae bore in the corm, reducing nutrient uptake and weakening the stability of the plant. Beauveria bassiana is especially important for controlling cryptic pests such as banana weevils. Various B. bassiana strains were isolated from banana weevil cadavers or soils in banana fields and have been tested in the laboratory. The most promising strains resulted in >90% larval mortality in 3 weeks. However, field efficacy of these promising strains against banana weevils is a key for development of a microbial control program. Various formulation methods (such as maize bran or millet waste) and their field delivery systems (using banana suckers, pseudostem traps or soil around the banana stems) have been tested. Results were variable and demonstrated that formulation methods and field delivery systems need to be integrated with prevailing agroecological conditions, such as soil types and management practices. Currently, pheromone- and kairomone-based traps are being investigated, alongside the use of B. bassiana as an artificial endophyte in banana tissue culture plants.

Contributed paper. Thursday, 4:45. 222
Effects of day versus evening application times on efficacy of Beauveria bassiana foliar sprays against Colorado potato beetle
Stephen P. Wright and Mark E. Ramos
USDA-ARS-PPRU, U.S. Plant, Soil, and Nutrition Laboratory, Tower Road, Ithaca, New York 14853, USA
Fungal pathogens are often applied during evening hours under the assumption that the favorable nighttime conditions of high humidity and moderate temperatures will enhance efficacy. Few studies, however, have actually tested this assumption. In a study conducted over four field seasons, we compared efficacy of Beauveria bassiana strain GHA treatments applied early to mid-day with treatments applied at sunset. Three or four sprays, each at the rate of 2.5 x 1013 viable conidia/ha, were applied at 3-4 day intervals using a tractor-mounted hydraulic sprayer. Each test employed a randomized complete-block design, with 5 replicate plots per treatment. Control of larvae was poor (usually < 30%); however, populations in the test generation adults were reduced 65-90%, depending on seasonal weather conditions. The day versus evening treatments produced means of 68.5 and 76.2% control, respectively, and the difference was
The entomopathogen *Beauveria bassiana* has been used in IPM programs to control the coffee berry borer (*Hypothenemus hampei*) in Colombia. Traditionally, development of biocontrol agents has involved the use of elite clonal strains. Instead, we explored the potential application of genetic diversity by determining the effect of strain mixtures on insect mortality. Cluster analysis grouped 11 strains of *Beauveria bassiana* in three genetic groups based on AFLPs, ITSs and β-tubulin characterization. The intraspecific genetic diversity of the strains was low but significant. The virulence of every strain towards the insect under lab conditions, using 1x10⁶ spores/ml, was between 89.91% and 57.5%. No differences were found when mixtures of genetically similar strains were tested, but mixtures of genetically different strains showed both, antagonism and synergism. A lowest virulence percentage (57%) was obtained by mixing the three most virulent strains of each group. The highest virulence percentage (93%) was obtained by mixing the three less virulent strains. The results indicate the promising potential of designing strain mixtures as an alternative for the biocontrol of *H. hampei* and other pests, and provides tools for the understanding of the ecological dynamics of entomopathogen populations under natural conditions.

Contributed paper. Thursday, 5:00. 223

**Use of genetic diversity in *Beauveria bassiana* for improving the biological control of the coffee berry borer**

Lina P. Cruz¹, Alvaro L. Gaitan², and Carmenza E. Gongora¹

¹Department of Entomology, and ²Department of Plant Pathology, CENICAFE (National Centre of Coffee Research). Chinchina, Caldas, Colombia

Microbial control studies were conducted with isolates of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from Ethiopia and South Africa against the spotted stem borer *Chilo partellus*. Four isolates of *B. bassiana* and six isolates of *M. anisopliae* were tested against second instar larva. Of these isolates, *B. bassiana* (BB-01) and *M. anisopliae* (PPRC-4, PPPRC-19, PPPRC-16 and EE-01) were found to be highly pathogenic inducing 90 to 100% mortality seven days after treatment. In subsequent assays, the fungal isolates were tested against third, fourth, fifth and sixth instar larvae. Second and sixth instar larvae were more susceptible to these isolates than third, fourth and fifth instar larvae. At 25 and 30°C the isolates induced 100% mortality to second instar larvae within four to six days. Treatment with the fungi was associated with a reduction in mean daily food consumption. In greenhouse trials, conidial suspension of 2 x 10⁶ conidia/ml of the pathogenic isolates was sprayed on 3 to 4 week-old maize plants infested with 20 second instar larvae per plant. Treatment with the fungi reduced foliar damage, stem tunneling and dead-heart formation.

Contributed paper. Thursday, 5:30. 225

**Microbial control of the spotted stem borer *Chilo partellus* with *Beauveria bassiana* and *Metarhizium anisopliae* from Ethiopia and South Africa**

Tadele Tefera

Alemaya University, Department of Plant Sciences, PO Box 42, Ethiopia

The application of *Metarhizium anisopliae* and *Beauveria bassiana* for the control of the longicorn beetle borer *Agrionome spinicollis* (Cerambycidae) in pecan trees

Ian R. Newton¹ and Andrew Ward²

¹Stahmann Farms, Trawalla, MSF 2058, Pallamallawa, NSW 2399, Australia, ²Becker Underwood, RMB 1084, Pacific Hwy, Somersby, NSW 2250, Australia

*Agrionome spinicollis* (Cerambycidae) is a serious pest of pecan trees in Australia. The beetle larvae bore into the heartwood of the trees causing eventual limb loss and in some cases whole tree loss. The adult beetles emerge and lay their eggs within a restricted period of 4 to 6 weeks in summer. The beetles lay their eggs deep within crevices in the trees with only one cohort of neonates present at the same time each year. This predictable behaviour makes them amendable to neonate control with mycoinsecticides. Sprays can be targeted at neonates with a minimum number of applications and the fungus is likely to persist in the dark crevices where eggs are deposited. A broad acre spray application of *M. anisopliae* from ground rigs was tested with the aim of targeting neonate larvae. Using caged beetles to establish eggs, this method resulted an 82% reduction in neonate establishment. Trials are currently in progress to determine optimal application timing, dosage and longevity. In other trials, *M. anisopliae* and *B. bassiana* strains were field tested by directly injecting oil suspensions into the borer galleries and by external application with a paintbrush. These methods were aimed at controlling the larger established larvae.
**Buses:** Shuttles will be provided between Guest Housing (The Commons) and Lucy Cuddy Center for those who do not wish to walk. Schedules will be posted in the Halls, Commons and Lucy Cuddy Center. Buses to the BBQ (Tuesday) and the Banquet (Thursday) will also leave from The Commons. Buses for the excursion (Tuesday) will leave from the Cuddy Center.